

* * * * * STN Columbus * * * * *

FILE 'HOME' ENTERED AT 17:37:59 ON 31 JAN 2003

=> fil .bec
COST IN U.S. DOLLARS

SINCE FILE
ENTRY
0.21

TOTAL
SESSION
0.21

FULL ESTIMATED COST

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 17:38:14 ON 31 JAN 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s (humaniz? or synthetic) (8a)gene/q
FILE 'MEDLINE'

1876 HUMANIZ?
123307 SYNTHETIC
L1 8414 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'SCISEARCH'

1807 HUMANIZ?
132520 SYNTHETIC
L2 6079 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'LIFESCI'

572 HUMANIZ?
35621 SYNTHETIC
L3 4094 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'BIOTECHDS'

1496 HUMANIZ?
10433 SYNTHETIC
L4 2478 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'BIOSIS'

1782 HUMANIZ?
181937 SYNTHETIC
L5 8402 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'EMBASE'

1580 HUMANIZ?
94833 SYNTHETIC
L6 6376 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'HCAPLUS'

3213 HUMANIZ?
496811 SYNTHETIC
L7 13448 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'NTIS'

130 HUMANIZ?
18328 SYNTHETIC
L8 161 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'ESBIOBASE'

838 HUMANIZ?
31050 SYNTHETIC
L9 2703 (HUMANIZ? OR SYNTHETIC) (8A)GENE/Q

FILE 'BIOTECHNO'

726 HUMANIZ?

09/645-708

L10 38588 SYNTHETIC
 5033 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

 FILE 'WPIDS'
 1401 HUMANIZ?
 190473 SYNTHETIC
 L11 1607 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

 TOTAL FOR ALL FILES
 L12 58795 (HUMANIZ? OR SYNTHETIC) (8A) GENE/Q

 => s l12 and codon preference
 FILE 'MEDLINE'
 30144 CODON
 27156 PREFERENCE
 83 CODON PREFERENCE
 (CODON (W) PREFERENCE)
 L13 9 L1 AND CODON PREFERENCE

 FILE 'SCISEARCH'
 20941 CODON
 35268 PREFERENCE
 78 CODON PREFERENCE
 (CODON (W) PREFERENCE)
 L14 6 L2 AND CODON PREFERENCE

 FILE 'LIFESCI'
 12569 "CODON"
 14499 "PREFERENCE"
 58 CODON PREFERENCE
 ("CODON" (W) "PREFERENCE")
 L15 5 L3 AND CODON PREFERENCE

 FILE 'BIOTECHDS'
 3516 CODON
 613 PREFERENCE
 12 CODON PREFERENCE
 (CODON (W) PREFERENCE)
 L16 3 L4 AND CODON PREFERENCE

 FILE 'BIOSIS'
 25162 CODON
 43180 PREFERENCE
 97 CODON PREFERENCE
 (CODON (W) PREFERENCE)
 L17 7 L5 AND CODON PREFERENCE

 FILE 'EMBASE'
 23589 "CODON"
 26080 "PREFERENCE"
 62 CODON PREFERENCE
 ("CODON" (W) "PREFERENCE")
 L18 7 L6 AND CODON PREFERENCE

 FILE 'HCAPLUS'
 29728 CODON
 31929 PREFERENCE
 118 CODON PREFERENCE
 (CODON (W) PREFERENCE)
 L19 13 L7 AND CODON PREFERENCE

 FILE 'NTIS'
 85 CODON
 2800 PREFERENCE

```

        1 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L20      0 L8 AND CODON PREFERENCE

FILE 'ESBIOBASE'
        11636 CODON
        11211 PREFERENCE
        32 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L21      4 L9 AND CODON PREFERENCE

FILE 'BIOTECHNO'
        20379 CODON
        5842 PREFERENCE
        60 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L22      7 L10 AND CODON PREFERENCE

FILE 'WPIDS'
        1815 CODON
        3444 PREFERENCE
        4 CODON PREFERENCE
          (CODON (W) PREFERENCE)
L23      1 L11 AND CODON PREFERENCE

TOTAL FOR ALL FILES
L24      62 L12 AND CODON PREFERENCE

=> s l12 and (transcription(3a) (regulat? or factor#) or splice or promoter# or
poly(w) 'a' or polyadenylat?)
FILE 'MEDLINE'
        192819 TRANSCRIPTION
        596604 REGULAT?
        1940904 FACTOR#
        78442 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
        10603 SPLICE
        95296 PROMOTER#
        47359 POLY
        6975728 'A'
        14982 POLY (W) 'A'
        6249 POLYADENYLAT?
L25      1491 L1 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
          PROMOTER# OR POLY (W) 'A' OR POLYADENYLAT?)

FILE 'SCISEARCH'
        150918 TRANSCRIPTION
        511475 REGULAT?
        1086286 FACTOR#
        63427 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
        11447 SPLICE
        97553 PROMOTER#
        132603 POLY
        8439122 'A'
        7044 POLY (W) 'A'
        4776 POLYADENYLAT?
L26      768 L2 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
          PROMOTER# OR POLY (W) 'A' OR POLYADENYLAT?)

FILE 'LIFESCI'
        82339 TRANSCRIPTION
        208401 REGULAT?
        257492 FACTOR#
        33323 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
        5542 SPLICE

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56034 PROMOTER#
 15831 POLY
 1862565 'A'
 5669 POLY(W) 'A'
 3875 POLYADENYLAT?
 L27 668 L3 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
 PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

 FILE 'BIOTECHDS'
 9993 TRANSCRIPTION
 19803 REGULAT?
 27604 FACTOR#
 1824 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
 760 SPLICE
 25157 PROMOTER#
 5083 POLY
 282006 'A'
 1548 POLY(W) 'A'
 1201 POLYADENYLAT?
 L28 765 L4 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
 PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

 FILE 'BIOSIS'
 179189 TRANSCRIPTION
 656960 REGULAT?
 1086428 FACTOR#
 64050 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
 11297 SPLICE
 107007 PROMOTER#
 133727 POLY
 7093953 'A'
 11465 POLY(W) 'A'
 7070 POLYADENYLAT?
 L29 1175 L5 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
 PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

 FILE 'EMBASE'
 170614 TRANSCRIPTION
 493807 REGULAT?
 962926 FACTOR#
 73866 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
 9307 SPLICE
 79412 PROMOTER#
 41232 POLY
 6149896 'A'
 8142 POLY(W) 'A'
 6416 POLYADENYLAT?
 L30 829 L6 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
 PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

 FILE 'HCAPLUS'
 211112 TRANSCRIPTION
 741065 REGULAT?
 1211776 FACTOR#
 104575 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
 12939 SPLICE
 147348 PROMOTER#
 563923 POLY
 16832871 'A'
 16965 POLY(W) 'A'
 9803 POLYADENYLAT?
 L31 2521 L7 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
 PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)


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FILE 'NTIS'
    1420 TRANSCRIPTION
    80107 REGULAT?
    142974 FACTOR#
        365 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
        444 SPLICE
        1456 PROMOTER#
        5475 POLY
    1615617 'A'
        64 POLY(W) 'A'
        9 POLYADENYLAT?
L32    11 L8 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
        PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'ESBIOBASE'
    83178 TRANSCRIPTION
    276433 REGULAT?
    322245 FACTOR#
    40587 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
    6483 SPLICE
    49759 PROMOTER#
    12819 POLY
    1732392 'A'
    2830 POLY(W) 'A'
    2252 POLYADENYLAT?
L33    420 L9 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
        PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'BIOTECHNO'
    140959 TRANSCRIPTION
    242763 REGULAT?
    265012 FACTOR#
    56923 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
    8068 SPLICE
    68955 PROMOTER#
    20039 POLY
    1335652 'A'
    6115 POLY(W) 'A'
    5578 POLYADENYLAT?
L34    831 L10 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
        PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

FILE 'WPIDS'
    8950 TRANSCRIPTION
    327624 REGULAT?
    126843 FACTOR#
    1995 TRANSCRIPTION(3A) (REGULAT? OR FACTOR#)
    8421 SPLICE
    26764 PROMOTER#
    144643 POLY
    1122622 'A'
    183 POLY(W) 'A'
    631 POLYADENYLAT?
L35    347 L11 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
        PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

TOTAL FOR ALL FILES
L36    9826 L12 AND (TRANSCRIPTION(3A) (REGULAT? OR FACTOR#) OR SPLICE OR
        PROMOTER# OR POLY(W) 'A' OR POLYADENYLAT?)

=> s l36 and (increas? or high) (2a) express? and muta?
FILE 'MEDLINE'
    1693180 INCREAS?
    1093760 HIGH

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730066 EXPRESS?
 57528 (INCREAS? OR HIGH) (2A) EXPRESS?
 395494 MUTA?
 L37 34 L25 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'SCISEARCH'
 1622611 INCREAS?
 1559810 HIGH
 919028 EXPRESS?
 54962 (INCREAS? OR HIGH) (2A) EXPRESS?
 371692 MUTA?
 L38 22 L26 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'LIFESCI'
 436209 INCREAS?
 305580 HIGH
 307082 EXPRESS?
 22374 (INCREAS? OR HIGH) (2A) EXPRESS?
 179100 MUTA?
 L39 15 L27 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'BIOTECHDS'
 51600 INCREAS?
 55265 HIGH
 88440 EXPRESS?
 4747 (INCREAS? OR HIGH) (2A) EXPRESS?
 32284 MUTA?
 L40 18 L28 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'BIOSIS'
 1848769 INCREAS?
 1237349 HIGH
 894578 EXPRESS?
 59827 (INCREAS? OR HIGH) (2A) EXPRESS?
 443327 MUTA?
 L41 27 L29 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'EMBASE'
 1606453 INCREAS?
 1063460 HIGH
 665361 EXPRESS?
 52172 (INCREAS? OR HIGH) (2A) EXPRESS?
 322831 MUTA?
 L42 15 L30 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'HCAPLUS'
 3431158 INCREAS?
 3112200 HIGH
 902850 EXPRESS?
 57360 (INCREAS? OR HIGH) (2A) EXPRESS?
 404571 MUTA?
 L43 37 L31 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'NTIS'
 175955 INCREAS?
 313715 HIGH
 36119 EXPRESS?
 512 (INCREAS? OR HIGH) (2A) EXPRESS?
 9249 MUTA?
 L44 0 L32 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

 FILE 'ESBIOBASE'
 537527 INCREAS?
 345902 HIGH

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380205 EXPRESS?
33512 (INCREAS? OR HIGH) (2A) EXPRESS?
180687 MUTA?
L45      10 L33 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

FILE 'BIOTECHNO'
350043 INCREAS?
273999 HIGH
406491 EXPRESS?
34445 (INCREAS? OR HIGH) (2A) EXPRESS?
219784 MUTA?
L46      15 L34 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

FILE 'WPIDS'
1072498 INCREAS?
1652039 HIGH
81119 EXPRESS?
3063 (INCREAS? OR HIGH) (2A) EXPRESS?
20195 MUTA?
L47      7 L35 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

TOTAL FOR ALL FILES
L48      200 L36 AND (INCREAS? OR HIGH) (2A) EXPRESS? AND MUTA?

=> s 124 or 148
FILE 'MEDLINE'
L49      43 L13 OR L37

FILE 'SCISEARCH'
L50      28 L14 OR L38

FILE 'LIFESCI'
L51      20 L15 OR L39

FILE 'BIOTECHDS'
L52      21 L16 OR L40

FILE 'BIOSIS'
L53      34 L17 OR L41

FILE 'EMBASE'
L54      22 L18 OR L42

FILE 'HCAPLUS'
L55      50 L19 OR L43

FILE 'NTIS'
L56      0 L20 OR L44

FILE 'ESBIOBASE'
L57      14 L21 OR L45

FILE 'BIOTECHNO'
L58      22 L22 OR L46

FILE 'WPIDS'
L59      8 L23 OR L47

TOTAL FOR ALL FILES
L60      262 L24 OR L48

=> dup rem 160
PROCESSING COMPLETED FOR L60
L61      103 DUP REM L60 (159 DUPLICATES REMOVED)

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=> d tot

L61 ANSWER 1 OF 103 MEDLINE
TI Two CCAAT/enhancer binding protein sites in the cytochrome P4503A1 locus.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (2003 Feb) 270 (3) 556-64.
Journal code: 0107600. ISSN: 0014-2956.
AU Rodrigues Elsa; Vilarem Marie-Jose; Ribeiro Vera; Maurel Patrick; Lechner Maria C
AN 2003036356 IN-PROCESS

L61 ANSWER 2 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Facilitating production of a protein for analyzing, designing and/or modifying an agent that can interact with a viral F protein, comprises expressing a nucleic acid optimized for expression of the protein, using a eukaryotic cell;
vector-mediated gene transfer and expression in host cell for recombinant vaccine and gene therapy
AU MASON A J; TUCKER S P; YOUNG P R
AN 2003-01543 BIOTECHDS
PI WO 2002042326 30 May 2002

L61 ANSWER 3 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel inducible expression system for regulating nucleic acid expression in gene therapy, provides undetectable biological effect or gene expression in absence of inducer, and **high expression** in presence of inducer;
vector expression in host cell, and electroporation use in disease therapy and gene therapy
AU ABRUZZESE R V; MEHTA V; NORDSTROM J L
AN 2003-00547 BIOTECHDS
PI WO 2002024899 28 Mar 2002

L61 ANSWER 4 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel human cytomegalovirus Intron A fragment for use in expression constructs, lacks full-length Intron A sequence, and enhance expression levels when present in expression constructs;
vector plasmid pCMVII-mediated recombinant protein gene transfer and expression in host cell for use in cancer diagnosis, prevention, therapy and gene therapy
AU THUDIUM K; SELBY M; ULMER J
AN 2002-16517 BIOTECHDS
PI WO 2002031137 18 Apr 2002

L61 ANSWER 5 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Screening for modulators of nuclear hormone receptor CAR, comprises administering a compound to a mouse expressing CAR and measuring induction of the CAR gene or measuring CAR gene expression in the presence and absence of the compound;
drug screening, transgenic animal model, cell culture transfection and reporter gene expression
AU MOORE D D; WEI P; CHUA S S
AN 2002-13289 BIOTECHDS
PI WO 2002025270 28 Mar 2002

L61 ANSWER 6 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Recombinant polypeptide for immunizing a subject, comprises non-overlapping segments of amino acids identical to cytokine receptor sequences;
recombinant cytokine receptor and antibody for use in recombinant vaccine, therapy and gene therapy
AU PARHAM C L; GORMAN D M; KURATA H; ARAI N; SANA T R; MATTSON J D; MURPHY E E; SAVKOOR C; GREIN J; SMITH K M; MCCLANAHAN T K
AN 2002-13069 BIOTECHDS

PI WO 2002020569 14 Mar 2002

L61 ANSWER 7 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel nucleic acid comprising regions of rat progression elevated gene-3
promoter for expressing foreign DNA in a host cell and treating
melanoma, neuroblastoma, astrocytoma, cervical or breast cancer in
humans;

retro virus vector-mediated gene transfer and expression in host for
use in melanoma, neuroblastoma, cervical, lung, prostate, colon and
glioblastoma cancer diagnostic, gene therapy and peptidomics

AU FISHER P B; SU Z

AN 2002-11241 BIOTECHDS

PI WO 2002008242 31 Jan 2002

L61 ANSWER 8 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Synthesizing DNA sequences using a series of overlapping template
oligonucleotides that have sequences corresponding to one strand of a DNA
sequence and which are incapable of extension in the synthesis;

vector-mediated gene transfer, expression in host cell and template
DNA for DNA synthesis, genomics, nucleic acid vaccine and gene therapy

AU MCALISTER M; SAVVA R; BHATTACHARYYA U

AN 2002-17947 BIOTECHDS

PI WO 2002050094 27 Jun 2002

L61 ANSWER 9 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI A synthetic DNA encoding an orange seapen *Ptiloscarcus gurneyi*-derived
green fluorescent protein with **codon preference** of
mammalian expression systems and biosensors

SO PCT Int. Appl., 77 pp.

CODEN: PIXXD2

IN Chen, Yih-Tai; Cao, Longguang

AN 2002:964489 HCAPLUS

DN 138:34138

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| WO 2002101020 | A2 | 20021219 | WO 2002-US18874 | 20020610 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

| | | | | |
|---------------|----|----------|----------------|----------|
| US 2002197673 | A1 | 20021226 | US 2001-977897 | 20011015 |
|---------------|----|----------|----------------|----------|

L61 ANSWER 10 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI An GHRH (somatoliberin) expression system inducible by a ligand-specific
Gene-Switch regulator protein and therapeutic uses

SO PCT Int. Appl., 90 pp.

CODEN: PIXXD2

IN Nordstrom, Jeffrey L.; Draghia-Akli, Ruxandra

AN 2002:927602 HCAPLUS

DN 138:20483

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| WO 2002097099 | A1 | 20021205 | WO 2001-US17573 | 20010530 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

L61 ANSWER 11 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI Modified vaccinia Ankara expressing modified HIV env, gag, and pol genes
and uses in vaccination
SO PCT Int. Appl., 112 pp.
CODEN: PIXXD2
IN Moss, Bernard; Wyatt, Linda; Earl, Patricia
AN 2002:716417 HCAPLUS
DN 137:246528

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 2002072754 | A2 | 20020919 | WO 2002-US6713 | 20020301 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |

L61 ANSWER 12 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI Site saturation **mutagenesis** and polynucleotide reassembly and
end selection in directed evolution
SO U.S., 114 pp., Cont.-in-part of U.S. Ser. No. 498,557.
CODEN: USXXAM
IN Short, Jay M.; Frey, Gerhard Johann
AN 2002:213739 HCAPLUS
DN 136:258264

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| US 6358709 | B1 | 20020319 | US 2000-522289 | 20000309 |
| US 5939250 | A | 19990817 | US 1996-651568 | 19960522 |
| US 5965408 | A | 19991012 | US 1996-677112 | 19960709 |
| US 5830696 | A | 19981103 | US 1996-760489 | 19961205 |
| US 6489145 | B1 | 20021203 | US 1997-962504 | 19971031 |
| US 6335179 | B1 | 20020101 | US 1998-185373 | 19981103 |
| US 6171820 | B1 | 20010109 | US 1999-246178 | 19990204 |
| US 6238884 | B1 | 20010529 | US 1999-267118 | 19990309 |
| US 6352842 | B1 | 20020305 | US 1999-276860 | 19990326 |
| US 6479258 | B1 | 20021112 | US 2000-495052 | 20000131 |
| US 6361974 | B1 | 20020326 | US 2000-535754 | 20000327 |
| US 2002086279 | A1 | 20020704 | US 2001-875412 | 20010606 |
| US 2002146762 | A1 | 20021010 | US 2001-885551 | 20010619 |

L61 ANSWER 13 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI Genetic engineering of *Aspergillus awamori* for production of bovine
chymosin (rennin)
SO Eur. Pat. Appl., 21 pp.
CODEN: EPXXDW
IN Elena Cardoza, Rosa; Gutierrez Martin, Santiago; Moralejo Lorenzo,
Francisco J.; Casqueiro Blanco, Francisco Javier; Martin Martin, Juan
Francisco
AN 2002:611731 HCAPLUS
DN 137:168388

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|----------|-----------------|----------|
| EP 1231272 | A2 | 20020814 | EP 2002-380019 | 20020130 |
| EP 1231272 | A3 | 20021113 | | |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

L61 ANSWER 14 OF 103 WPIDS (C) 2003 THOMSON DERWENT
TI New isolated nucleic acid representing a **synthetic BAX-gene**, useful as medicament for treating, preventing and/or alleviating yeast or fungal infections or proliferative disorders, or for preventing apoptosis in certain diseases.

PI WO 2002064766 A2 20020822 (200271)* EN 344p C12N015-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO
RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
IN CONTRERAS, R H; EBERHARDT, I; LUYTEN, W H M L; REEKMANS, R J

L61 ANSWER 15 OF 103 WPIDS (C) 2003 THOMSON DERWENT
TI Novel inducible expression system for regulating nucleic acid expression in gene therapy, provides undetectable biological effect or gene expression in absence of inducer, and **high expression** in presence of inducer.

PI WO 2002024899 A2 20020328 (200257)* EN 101p C12N015-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO
RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001096354 A 20020402 (200257) C12N015-00
IN ABRUZZESE, R V; MEHTA, V; NORDSTROM, J L

L61 ANSWER 16 OF 103 WPIDS (C) 2003 THOMSON DERWENT
TI Producing a recombinant adenoviral vector library useful for screening gene function, by transfecting a recombinant nucleic acid, and optionally adapter plasmid, into a cell with adenoviral E1-complementing sequences.

PI US 6413776 B1 20020702 (200272)* 62p C12N005-10
IN BOUT, A; SHOUTEN, G; VAN ES, H H G; VOGELS, R

L61 ANSWER 17 OF 103 MEDLINE DUPLICATE 1
TI Novel baculovirus DNA elements strongly stimulate activities of exogenous and endogenous **promoters**.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Feb 15) 277 (7) 5256-64.
Journal code: 2985121R. ISSN: 0021-9258.
AU Lo Huei-Ru; Chou Cheng-Chung; Wu Tzong-Yuan; Yuen Joyce Pui-Yee; Chao Yu-Chan
AN 2002106153 MEDLINE

L61 ANSWER 18 OF 103 MEDLINE
TI Multiple pulmonary adenomas in the lung of transgenic mice overexpressing the RON receptor tyrosine kinase. Recepteur d'origine nantais.

SO CARCINOGENESIS, (2002 Nov) 23 (11) 1811-9.
Journal code: 8008055. ISSN: 0143-3334.
AU Chen Yi-Qing; Zhou Yong-Qing; Fu Lu-Hong; Wang Dong; Wang Ming-Hai
AN 2002684032 MEDLINE

L61 ANSWER 19 OF 103 MEDLINE DUPLICATE 2
TI Long-term and tight control of gene expression in mouse skeletal muscle by a new hybrid human **transcription factor**.

SO MOLECULAR THERAPY, (2002 Nov) 6 (5) 653-63.
Journal code: 100890581. ISSN: 1525-0016.
AU Roscilli Giuseppe; Rinaudo Cira Daniela; Cimino Monica; Sporeno Elisabetta; Lamartina Stefania; Ciliberto Gennaro; Toniatti Carlo
AN 2002650520 IN-PROCESS

L61 ANSWER 20 OF 103 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
 TI Tetracycline-inducible stable mammalian cell line **expression** systems for **high-level expression** of toxic rhodopsin **mutants**, non-glycosylated rhodopsin, and rhodopsin containing defined N-glycans.
 SO FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A150.
<http://www.fasebj.org/>. print.
 Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
 ISSN: 0892-6638.
 AU Reeves, Philip J. (1); Callewaert, Nico; Contreras, Roland; Kim, Jong-Myoung (1); Khorana, H. Gobind (1)
 AN 2002:323154 BIOSIS

L61 ANSWER 21 OF 103 WPIDS (C) 2003 THOMSON DERWENT
 TI Diagnosing and treating glaucoma by analysis and control of the Frizzled Related Protein and Wnt pathway protein expression and activity.
 PI WO 2001064949 A2 20010907 (200165)* EN 74p C12Q001-68
 RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
 W: AU BR CA CN JP KR MX PL ZA
 AU 2001041768 A 20010912 (200204) C12Q001-68
 US 2002049177 A1 20020425 (200233) A61K048-00
 EP 1259648 A2 20021127 (200302) EN C12Q001-68
 R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
 IN CLARK, A F; FINGERT, J; MCNATT, L; STONE, E; WANG, W; STONE, E M

L61 ANSWER 22 OF 103 WPIDS (C) 2003 THOMSON DERWENT
 TI New isolated **mutated** human p53 polypeptides for inducing toxicity in a cell, treating cancer and identifying compounds that mimic toxic or supertransactivating **mutations**.
 PI WO 2001009325 A2 20010208 (200113)* EN 144p C12N015-12
 RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
 W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
 AU 2000062395 A 20010219 (200129) C12N015-12
 EP 1204745 A2 20020515 (200239) EN C12N015-12
 R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
 IN INGA, A; RESNICK, M A

L61 ANSWER 23 OF 103 MEDLINE DUPLICATE 3
 TI Deletion of RAR carboxyl terminus reveals **promoter-** and receptor-specific AF-1 effects.
 SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2001 Dec 21) 289 (5) 950-6.
 Journal code: 0372516. ISSN: 0006-291X.
 AU Aneskievich B J
 AN 2001700851 MEDLINE

L61 ANSWER 24 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)DUPLICATE 4
 TI A rapid and simple method for construction and expression of a **synthetic** human growth hormone **gene** in Escherichia coli
 SO JOURNAL OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, (30 NOV 2001) Vol. 34, No. 6, pp. 502-508.
 Publisher: SPRINGER-VERLAG SINGAPORE PTE LTD, #04-01 CENCON I, 1 TANNERY RD, SINGAPORE 347719, SINGAPORE.
 ISSN: 1225-8687.
 AU Roytrakul S; Eurwilaichitr L (Reprint); Suprasongsin C; Panyim S
 AN 2001:961866 SCISEARCH

L61 ANSWER 25 OF 103 MEDLINE DUPLICATE 5
 TI Overexpression of artificial **synthetic gene** of
 Aspergillus niger NRRL3135 phytase in Pichia pastoris.
 SO SHENG WU KUNG CH ENG HSUEH PAO, (2001 May) 17 (3) 254-8.
 Journal code: 9426463. ISSN: 1000-3061.
 AU Bei J L; Chen Z; Yang L; Liao L; Wang X Z; Jiang Z Y
 AN 2001474246 MEDLINE

L61 ANSWER 26 OF 103 MEDLINE DUPLICATE 6
 TI Reduction of wobble-position GC bases in Corynebacteria genes and
 enhancement of PCR and heterologous expression.
 SO JOURNAL OF MOLECULAR MICROBIOLOGY AND BIOTECHNOLOGY, (2001 Jan) 3 (1)
 123-6.
 Journal code: 100892561. ISSN: 1464-1801.
 AU Sanli G; Blaber S I; Blaber M
 AN 2001459358 MEDLINE

L61 ANSWER 27 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
 TI Modified **synthetic DNA sequences** comprise
 modification of the truncated cry9Aa **gene** of Bacillus
 thuringiensis for improved insect control in plants;
 transgenic plant construction with improved disease-resistance
 AU Kuvshinov V; Kanerva A; Koivu K; Pehu E
 AN 2000-06780 BIOTECHDS
 PI WO 2000011025 2 Mar 2000

L61 ANSWER 28 OF 103 HCAPLUS COPYRIGHT 2003 ACS
 TI CDX2 is downstream mediator of APC tumor suppressor activity
 SO PCT Int. Appl., 27 pp.
 CODEN: PIXXD2
 IN Dacosta, Luis; Vogelstein, Bert; Kinzler, Kenneth W.; He, Tong-chuan
 AN 2000:824448 HCAPLUS
 DN 134:1380

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| WO 2000070089 | A1 | 20001123 | WO 2000-US12893 | 20000512 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 6511799 | B1 | 20030128 | US 1999-311551 | 19990514 |

L61 ANSWER 29 OF 103 HCAPLUS COPYRIGHT 2003 ACS
 TI Generation of genetic vaccines and immunomodulatory polynucleotides by
 non-stochastic directed evolution techniques
 SO PCT Int. Appl., 718 pp.
 CODEN: PIXXD2
 IN Short, Jay M.
 AN 2000:553678 HCAPLUS
 DN 133:160529

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| WO 2000046344 | A2 | 20000810 | WO 2000-US3086 | 20000204 |
| WO 2000046344 | A3 | 20001228 | | |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, | | | | |

BY, KG, KZ, MD, RU, TJ, TM
 RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,
 DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF,
 CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
 US 6171820 B1 20010109 US 1999-246178 19990204
 EP 1073710 A2 20010207 EP 2000-913378 20000204
 EP 1073710 A3 20010307
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO
 US 2002086279 A1 20020704 US 2001-875412 20010606

L61 ANSWER 30 OF 103 WPIDS (C) 2003 THOMSON DERWENT
 TI Modified **synthetic** DNA **sequences** comprise modification
 of the truncated cry9Aa **gene** of Bacillus thuringiensis for
 improved insect control in plants.
 PI WO 2000011025 A1 20000302 (200019)* EN 90p C07K014-325
 RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
 OA PT SD SE SL SZ UG ZW
 W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES
 FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
 LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ
 TM TR TT UA UG US UZ VN YU ZA ZW
 AU 9954244 A 20000314 (200031) C07K014-325
 EP 1107984 A1 20010620 (200135) EN C07K014-325
 R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
 RO SE SI
 CN 1326464 A 20011212 (200225) C07K014-325
 IN KANERVA, A; KOIVU, K; KUVSHINOV, V; PEHU, E

L61 ANSWER 31 OF 103 MEDLINE DUPLICATE 7
 TI Runt domain factor (Runx)-dependent effects on CCAAT/ enhancer-binding
 protein delta expression and activity in osteoblasts.
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jul 14) 275 (28) 21746-53.
 Journal code: 2985121R. ISSN: 0021-9258.
 AU McCarthy T L; Ji C; Chen Y; Kim K K; Imagawa M; Ito Y; Centrella M
 AN 2000396738 MEDLINE

L61 ANSWER 32 OF 103 MEDLINE DUPLICATE 8
 TI The Pezcoller lecture: cancer cell cycles revisited.
 SO CANCER RESEARCH, (2000 Jul 15) 60 (14) 3689-95. Ref: 118
 Journal code: 2984705R. ISSN: 0008-5472.
 AU Sherr C J
 AN 2000402340 MEDLINE

L61 ANSWER 33 OF 103 LIFESCI COPYRIGHT 2003 CSA
 TI CaMKII alpha -cre Transgene Expression and Recombination Patterns in the
 Mouse Brain
 SO Genesis, (20000200) vol. 26, no. 2, pp. 133-135. Special Issue: Issue
 Specific Expression of Cre Recombinase in Mice..
 ISSN: 1526-954X.
 AU Dragatsis, I.; Zeitlin, S.
 AN 2000:66593 LIFESCI

L61 ANSWER 34 OF 103 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
 TI Beyond the full-length **gene** barrier: The large-scale production
 of **synthetic genes**.
 SO International Genome Sequencing and Analysis Conference, (2000) Vol. 12,
 pp. 72. print.
 Meeting Info.: 12th International Genome Sequencing and Analysis
 Conference Miami Beach, Florida, USA September 12-15, 2000
 AU Kittle, Joseph D., Jr. (1)
 AN 2001:514716 BIOSIS

L61 ANSWER 35 OF 103 MEDLINE DUPLICATE 9

TI Codon optimization of xylanase gene xynB from the thermophilic bacterium
Dictyoglomus thermophilum for expression in the filamentous fungus
Trichoderma reesei.
SO FEMS MICROBIOLOGY LETTERS, (2000 Sep 1) 190 (1) 13-9.
Journal code: 7705721. ISSN: 0378-1097.
AU Te'o V S; Cziferszky A E; Bergquist P L; Nevalainen K M
AN 2001096599 MEDLINE

L61 ANSWER 36 OF 103 WPIDS (C) 2003 THOMSON DERWENT
TI New flowering locus T polypeptide that regulates flowering time,
particularly used to accelerate flowering.
PI WO 9953070 A1 19991021 (199952)* EN 63p C12N015-29
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU
LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR
TT UA UG UZ VN YU ZA ZW
AU 9935601 A 19991101 (200013)
EP 1073743 A1 20010207 (200109) EN C12N015-29
R: AT BE CH CY DE DK ES FI FR GB GR IE LI LU MC NL PT SE
US 6225530 B1 20010501 (200126) C12N015-29
CN 1302328 A 20010704 (200158) C12N015-29
BR 9910123 A 20011002 (200167) C12N015-29
KR 2001042756 A 20010525 (200168) C07K014-415
US 2001049831 A1 20011206 (200203) C12N005-02
US 2002029395 A1 20020307 (200221) C12N015-82
JP 2002511270 W 20020416 (200242) 62p C12N015-09
IN WEIGEL, D; KARDAILSKY, I

L61 ANSWER 37 OF 103 WPIDS (C) 2003 THOMSON DERWENT
TI New human lectomedin receptor polypeptide, used to identify specific
binding partners for treating e.g. vascular disease.
PI WO 9945111 A1 19990910 (199948)* EN 166p C12N015-12
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SL SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD
GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT
UA UG UZ VN YU ZW
AU 9929807 A 19990920 (200007) C12N015-12
EP 1060248 A1 20001220 (200105) EN C12N015-12
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
JP 2002505104 W 20020219 (200216) 174p C12N015-09
US 6479256 B1 20021112 (200278) C12P021-06
IN HAYFLICK, J S

L61 ANSWER 38 OF 103 MEDLINE
TI Development of viral vectors for gene therapy of beta-chain
hemoglobinopathies: optimization of a gamma-globin gene expression
cassette.
SO BLOOD, (1999 Apr 1) 93 (7) 2208-16.
Journal code: 7603509. ISSN: 0006-4971.
AU Li Q; Emery D W; Fernandez M; Han H; Stamatoyannopoulos G
AN 1999192436 MEDLINE

L61 ANSWER 39 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI Study on the expression of recombinant human thrombopoietin in Escherichia
coli
SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1999), 15(1), 19-23
CODEN: ZSHXF2; ISSN: 1007-7626
AU Zhao, Dong; Lu, Baisong; Chen, Lin; Liu, Xiaolan; Huang, Peitang
AN 1999:202170 HCAPLUS
DN 131:1231

L61 ANSWER 40 OF 103 MEDLINE DUPLICATE 10
 TI Gene synthesis by a LCR-based approach: high-level production of leptin-L54 using **synthetic gene** in Escherichia coli.
 SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Jul 9) 248 (1) 200-3.
 Journal code: 0372516. ISSN: 0006-291X.
 AU Au L C; Yang F Y; Yang W J; Lo S H; Kao C F
 AN 1998340875 MEDLINE

L61 ANSWER 41 OF 103 LIFESCI COPYRIGHT 2003 CSA
 TI Transcriptional Regulation of the Human Proenkephalin Gene by Conformational Switching: Implications for Decoy Design
 SO Antisense Nucleic Acid Drug Dev., (19980400) vol. 8, no. 2, pp. 159-165. ISSN: 1087-2906.
 AU Spiro, C.; McMurray, C.T.*
 AN 1998:97956 LIFESCI

L61 ANSWER 42 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)DUPLICATE 11
 TI Instability of Aspergillus niger glucoamylase cDNA in a high copy number vector cloned into Escherichia coli
 SO ASIA-PACIFIC JOURNAL OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY, (JUN 1998) Vol. 6, No. 1, pp. 39-46.
 Publisher: UNIV MALAYA, INST POSTGRADUATE STUDIES & RESEARCH, KUALA LUMPUR 50603, MALAYSIA.
 ISSN: 0128-7451.
 AU Abdulrashid N (Reprint); Hartley B S
 AN 1998:677932 SCISEARCH

L61 ANSWER 43 OF 103 MEDLINE
 TI A point **mutation** within CD45 exon A is the cause of variant CD45RA splicing in humans.
 SO EUROPEAN JOURNAL OF IMMUNOLOGY, (1998 Jan) 28 (1) 22-9.
 Journal code: 1273201. ISSN: 0014-2980.
 AU Zilch C F; Walker A M; Timon M; Goff L K; Wallace D L; Beverley P C
 AN 1998143724 MEDLINE

L61 ANSWER 44 OF 103 HCAPLUS COPYRIGHT 2003 ACS
 TI **Synthetic HIV genes** for use in vector vaccines
 SO PCT Int. Appl., 82 pp.
 CODEN: PIXXD2
 IN Shiver, John W.; Davies, Mary-Ellen; Freed, Daniel C.; Liu, Margaret A.; Perry, Helen C.
 AN 1997:579828 HCAPLUS
 DN 127:247095

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9731115 | A2 | 19970828 | WO 1997-US2294 | 19970218 |
| WO 9731115 | A3 | 19971009 | | |
| W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| AU 9721246 | A1 | 19970910 | AU 1997-21246 | 19970218 |
| AU 729231 | B2 | 20010125 | | |
| EP 904380 | A2 | 19990331 | EP 1997-906594 | 19970218 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, SI, LT, LV, FI, RO | | | | |
| BR 9707672 | A | 19990413 | BR 1997-7672 | 19970218 |
| CN 1216064 | A | 19990505 | CN 1997-193817 | 19970218 |
| JP 2000505299 | T2 | 20000509 | JP 1997-530231 | 19970218 |

| | | | | |
|------------|---|----------|--------------|----------|
| ZA 9701449 | A | 19970822 | ZA 1997-1449 | 19970220 |
| NO 9803876 | A | 19981021 | NO 1998-3876 | 19980821 |

L61 ANSWER 45 OF 103 HCAPLUS COPYRIGHT 2003 ACS
 TI Recombinant nucleic acids containing a negative transdominant
mutant of gene rev for inhibiting HIV gene expression
 SO U.S., 35 pp.
 CODEN: USXXAM
 IN Nabel, Gary J.; Yang, Zhi-yong; Liu, Jinsong; Woffendin, Clive
 AN 1997:492879 HCAPLUS
 DN 127:186605

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|------------|------|----------|-----------------|----------|
| | ----- | --- | ----- | ----- | ----- |
| PI | US 5650306 | A | 19970722 | US 1993-73836 | 19930607 |

L61 ANSWER 46 OF 103 HCAPLUS COPYRIGHT 2003 ACS
 TI Inhibitor-resistant urokinase and transgenic animals secreting the enzyme
 into the milk
 SO U.S., 16 pp., Cont. of U. S. Ser. No. 631,673, abandoned.
 CODEN: USXXAM
 IN Wei, Cha Mer
 AN 1997:492809 HCAPLUS
 DN 127:146512

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|------------|------|----------|-----------------|----------|
| | ----- | --- | ----- | ----- | ----- |
| PI | US 5648253 | A | 19970715 | US 1992-942157 | 19920908 |

L61 ANSWER 47 OF 103 MEDLINE DUPLICATE 12
 TI Specific sequence modifications of a cry3B endotoxin gene result in
high levels of **expression** and insect resistance.
 SO PLANT MOLECULAR BIOLOGY, (1997 Jun) 34 (3) 485-96.
 Journal code: 9106343. ISSN: 0167-4412.
 AU Iannacone R; Grieco P D; Cellini F
 AN 97369371 MEDLINE

L61 ANSWER 48 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)
 TI Proteolytic activity in vivo and encapsidation of recombinant human
 immunodeficiency virus type 1 proteinase expressed in baculovirus-infected
 cells
 SO JOURNAL OF GENERAL VIROLOGY, (JAN 1997) Vol. 78, Part 1, pp. 131-142.
 Publisher: SOC GENERAL MICROBIOLOGY, HARVEST HOUSE 62 LONDON ROAD,
 READING, BERKS, ENGLAND RG1 5AS.
 ISSN: 0022-1317.
 AU Royer M; Bardy M; Gay B; Tournier J; Boulanger P (Reprint)
 AN 97:91145 SCISEARCH

L61 ANSWER 49 OF 103 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.
 AN 1997119200 ESBIODASE
 TI Expression of de novo high-lysine .alpha.-helical coiled-coil proteins
 may significantly increase the accumulated levels of lysine in mature
 seeds of transgenic tobacco plants
 AU Keeler S.J.; Maloney C.L.; Webber P.Y.; Patterson C.; Hirata L.T.; Falco
 S.C.; Rice J.A.
 CS S.J. Keeler, Department of Plant/Soil Sciences, 149 Townsend Hall,
 University of Delaware, Newark, DE 19717, United States.
 SO Plant Molecular Biology, (1997), 34/1 (15-29), 55 reference(s)
 CODEN: PMBIDB ISSN: 0167-4412
 DT Journal; Article
 CY Netherlands
 LA English
 SL English

L61 ANSWER 50 OF 103 MEDLINE DUPLICATE 13
 TI Structure and function in rhodopsin: **high** level

expression of a synthetic bovine opsin gene
and its **mutants** in stable mammalian cell lines.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Oct 15) 93 (21) 11487-92.
Journal code: 7505876. ISSN: 0027-8424.

AU Reeves P J; Thurmond R L; Khorana H G
AN 97030221 MEDLINE

L61 ANSWER 51 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Cloning and characterization of cDNA encoding farnesyl-diphosphate-synthase from rubber tree;
potential crop improvement

SO Plant Mol.Biol.; (1996) 30, 5, 935-46
CODEN: PMBIDB ISSN: 0167-4412

AU Adiwilaga K; *Kush A
AN 1996-08810 BIOTECHDS

L61 ANSWER 52 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)DUPLICATE 14
TI Optimization of the bovine growth hormone gene expression in E-coli
SO MOLECULES AND CELLS, (31 DEC 1996) Vol. 6, No. 6, pp. 712-718.
Publisher: KOREAN SOC MOLECULAR BIOLOGY, KOREA SCI TECHNOLOGY CENTER, ROOM 815, 635-4 YEOGSAM-DONG KANGNAM-GU, SEOUL 135-703, SOUTH KOREA.
ISSN: 1016-8478.

AU Choi J W; Lee S Y (Reprint)
AN 97:33963 SCISEARCH

L61 ANSWER 53 OF 103 MEDLINE DUPLICATE 15
TI A novel Escherichia coli vector for oxygen-inducible **high level expression** of foreign genes.

SO GENE, (1996 Oct 17) 176 (1-2) 269-70.
Journal code: 7706761. ISSN: 0378-1119.

AU Gao B; Flores S C; Bose S K; McCord J M
AN 97075943 MEDLINE

L61 ANSWER 54 OF 103 MEDLINE DUPLICATE 16
TI Chemical synthesis of the Plasmodium falciparum dihydrofolate reductase-thymidylate synthase gene.

SO MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1996 Dec 2) 83 (1) 93-106.
Journal code: 8006324. ISSN: 0166-6851.

AU Prapunwattana P; Sirawaraporn W; Yuthavong Y; Santi D V
AN 97164122 MEDLINE

L61 ANSWER 55 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)
TI OVEREXPRESSION OF A NOVEL HIGHLY-ACTIVE TUMOR-NECROSIS-FACTOR **MUTANT** IN ESCHERICHIA-COLI

SO ACTA BIOCHIMICA ET BIOPHYSICA SINICA, (JAN 1996) Vol. 28, No. 1, pp. 49-55.
ISSN: 0582-9879.

AU GAO C S (Reprint); MAO S L; YU Y; CHEN C Q
AN 96:209988 SCISEARCH

L61 ANSWER 56 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI Gene Synthesis, **High-Level Expression**, and **Mutagenesis** of Thiobacillus ferrooxidans Rusticyanin: His 85 Is a Ligand to the Blue Copper Center

SO Biochemistry (1995), 34(20), 6640-8
CODEN: BICHAW; ISSN: 0006-2960

AU Casimiro, Danilo R.; Toy-Palmer, Anna; Blake, Robert C. II; Dyson, H. Jane
AN 1995:568826 HCAPLUS
DN 123:247906

L61 ANSWER 57 OF 103 MEDLINE
TI An Ets site in the whey acidic protein gene **promoter** mediates transcriptional activation in the mammary gland of pregnant mice but is

dispensable during lactation.

SO MOLECULAR ENDOCRINOLOGY, (1995 Jun) 9 (6) 717-24.
Journal code: 8801431. ISSN: 0888-8809.

AU McKnight R A; Spencer M; Dittmer J; Brady J N; Wall R J; Hennighausen L
AN 96174907 MEDLINE

L61 ANSWER 58 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)
TI OVEREXPRESSION, PURIFICATION, AND CHARACTERIZATION OF ESCHERICHIA-COLI
ACYL CARRIER PROTEIN AND 2 **MUTANT** PROTEINS
SO PROTEIN EXPRESSION AND PURIFICATION, (AUG 1995) Vol. 6, No. 4, pp.
394-400.
ISSN: 1046-5928.

AU HILL R B; MACKENZIE K R; FLANAGAN J M; CRONAN J E; PRESTEGARD J H
(Reprint)
AN 95:535140 SCISEARCH

L61 ANSWER 59 OF 103 MEDLINE
TI Plasmid **mutagenesis** by PCR for **high-level**
expression of para-hydroxybenzoate hydroxylase.
SO PROTEIN EXPRESSION AND PURIFICATION, (1995 Apr) 6 (2) 164-8.
Journal code: 9101496. ISSN: 1046-5928.

AU Moran G R; Entsch B
AN 95329936 MEDLINE

L61 ANSWER 60 OF 103 MEDLINE
TI Human chorionic somatomammotropin gene enhancer activity is dependent on
the blockade of a repressor mechanism.
SO MOLECULAR ENDOCRINOLOGY, (1994 Apr) 8 (4) 478-89.
Journal code: 8801431. ISSN: 0888-8809.

AU Lytras A; Cattini P A
AN 94329105 MEDLINE

L61 ANSWER 61 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)
TI IMPORTANCE OF **CODON PREFERENCE** FOR PRODUCTION OF HUMAN
RAP74 AND RECONSTITUTION OF THE RAP30/74 COMPLEX
SO PROTEIN EXPRESSION AND PURIFICATION, (OCT 1994) Vol. 5, No. 5, pp.
476-485.
ISSN: 1046-5928.

AU WANG B Q; LEI L; BURTON Z F (Reprint)
AN 94:626199 SCISEARCH

L61 ANSWER 62 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI ColE1-compatible vectors for **high-level expression** of
cloned DNAs from the T7 **promoter**;
phage T7-based plasmid pMR102, plasmid pMR103 and plasmid pMR101
construction
SO Gene; (1994) 144, 1, 59-62
CODEN: GENED6

AU Munson M; Predki P F; *Regan L
AN 1994-11445 BIOTECHDS

L61 ANSWER 63 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Synthetic eukaryotic **promoter**;
mutant mouse-mammary-tumor virus, hMT-IIA, hormone, metal,
heat shock, interferon, etc., inducible **promoter** for
high level gene expression in Vero, CHO, HeLa,
RatII, epithelium cell culture
AN 1993-14976 BIOTECHDS
PI WO 9320218 14 Oct 1993

L61 ANSWER 64 OF 103 MEDLINE DUPLICATE 17
TI The dihydrofolate reductase domain of Plasmodium falciparum thymidylate
synthase-dihydrofolate reductase. Gene synthesis, expression, and
anti-folate-resistant mutants.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Oct 15) 268 (29) 21637-44.
Journal code: 2985121R. ISSN: 0021-9258.

AU Sirawaraporn W; Prapunwattana P; Sirawaraporn R; Yuthavong Y; Santi D V
AN 94012742 MEDLINE

L61 ANSWER 65 OF 103 MEDLINE DUPLICATE 18
TI **Mutations** in the conserved loop of human U5 snRNA generate use
of novel cryptic 5' **splice** sites in vivo.

SO EMBO JOURNAL, (1993 Dec 15) 12 (13) 5181-9.
Journal code: 8208664. ISSN: 0261-4189.

AU Cortes J J; Sontheimer E J; Seiwert S D; Steitz J A
AN 94085393 MEDLINE

L61 ANSWER 66 OF 103 MEDLINE
TI Synthesis of the Escherichia coli K-12 nucleoid-associated DNA-binding
protein H-NS is subjected to growth-phase control and autoregulation.

SO MOLECULAR MICROBIOLOGY, (1993 May) 8 (5) 875-89.
Journal code: 8712028. ISSN: 0950-382X.

AU Dersch P; Schmidt K; Bremer E
AN 93360818 MEDLINE

L61 ANSWER 67 OF 103 MEDLINE DUPLICATE 19
TI HIV1 integrase expressed in Escherichia coli from a **synthetic**
gene.

SO GENE, (1993 Dec 22) 136 (1-2) 323-8.
Journal code: 7706761. ISSN: 0378-1119.

AU Holler T P; Foltin S K; Ye Q Z; Hupe D J
AN 94124023 MEDLINE

L61 ANSWER 68 OF 103 MEDLINE DUPLICATE 20
TI A model system for studying membrane biogenesis. Overexpression of
cytochrome b5 in yeast results in marked proliferation of the
intracellular membrane.

SO JOURNAL OF CELL SCIENCE, (1993 Sep) 106 (Pt 1) 249-59.
Journal code: 0052457. ISSN: 0021-9533.

AU Vergeres G; Yen T S; Aggeler J; Lausier J; Waskell L
AN 94095658 MEDLINE

L61 ANSWER 69 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)
TI THE INTRACELLULAR PRODUCTION AND SECRETION OF HIV-1 ENVELOPE PROTEIN IN
THE METHYLOTROPHIC YEAST PICHIA-PASTORIS

SO GENE, (22 DEC 1993) Vol. 136, No. 1-2, pp. 111-119.
ISSN: 0378-1119.

AU SCORER C A (Reprint); BUCKHOLZ R G; CLARE J J; ROMANOS M A
AN 94:104191 SCISEARCH

L61 ANSWER 70 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI Expression constructs for estrogen-inducible transcription and
stabilization of transcripts

SO PCT Int. Appl., 113 pp.
CODEN: PIXXD2

IN Shapiro, David J.; Nielsen, David A.; Kemper, Byron W.; Szczesna-Skorupa,
Elzbieta; Xing, Hong
AN 1992:209130 HCAPLUS
DN 116:209130

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| WO 9202529 | A1 | 19920220 | WO 1991-US5404 | 19910730 |
| W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, PL, RO, SD, SE, SU | | | | |
| RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG | | | | |
| AU 9184160 | A1 | 19920302 | AU 1991-84160 | 19910730 |

L61 ANSWER 71 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
 TI Human hemoglobin expression in Escherichia coli: importance of optimal
 codon usage;
 alpha-globin and beta-globin gene expression as an operon downstream
 of a lac **promoter**
 SO Biochemistry; (1992) 31, 36, 8619-28
 CODEN: BICHAW
 AU Hernan R A; Hui H L; Andracki M E; Noble R W; Sligar S G; *Walder R Y
 AN 1992-12988 BIOTECHDS

L61 ANSWER 72 OF 103 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
 TI CONSTRUCTION OF STABLE LAMB-SHIGA TOXIN B-SUBUNIT HYBRIDS ANALYSIS OF
 EXPRESSION IN SALMONELLA-TYPHIMURIUM AROA STRAINS AND STIMULATION OF
 B-SUBUNIT-SPECIFIC MUCOSAL AND SERUM ANTIBODY RESPONSES.
 SO INFECT IMMUN, (1992) 60 (8), 3345-3359.
 CODEN: INFIBR. ISSN: 0019-9567.
 AU SU G-F; BRAHMBHATT H U; WEHLAND J; ROHDE M; TIMMIS K N
 AN 1992:432979 BIOSIS

L61 ANSWER 73 OF 103 MEDLINE
 TI In vivo control of **promoter** and terminator efficiencies at a
 distance.
 SO MOLECULAR MICROBIOLOGY, (1992 Jun) 6 (12) 1681-91.
 Journal code: 8712028. ISSN: 0950-382X.
 AU Jacquet M A; Reiss C
 AN 92356832 MEDLINE

L61 ANSWER 74 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)
 TI CLONING AND **HIGH-LEVEL EXPRESSION** OF A
SYNTHETIC GENE FOR HUMAN BASIC FIBROBLAST GROWTH-FACTOR
 SO JOURNAL OF BIOTECHNOLOGY, (FEB 1992) Vol. 22, No. 3, pp. 299-310.
 ISSN: 0168-1656.
 AU MILEV P V; GEORGIEV O I; TZARNORETCHKI P O; HADJIOLOV A A (Reprint)
 AN 92:139370 SCISEARCH

L61 ANSWER 75 OF 103 MEDLINE DUPLICATE 21
 TI High-level production of active HIV-1 protease in Escherichia coli.
 SO GENE, (1992 Dec 15) 122 (2) 263-9.
 Journal code: 7706761. ISSN: 0378-1119.
 AU Rangwala S H; Finn R F; Smith C E; Berberich S A; Salsgiver W J; Stallings
 W C; Glover G I; Olins P O
 AN 93138395 MEDLINE

L61 ANSWER 76 OF 103 MEDLINE
 TI **Synthetic cryIIIA gene** from Bacillus thuringiensis
 improved for **high expression** in plants.
 SO TRANSGENIC RESEARCH, (1992 Sep) 1 (5) 228-36.
 Journal code: 9209120. ISSN: 0962-8819.
 AU Sutton D W; Havstad P K; Kemp J D
 AN 93244913 MEDLINE

L61 ANSWER 77 OF 103 MEDLINE DUPLICATE 22
 TI Expression of **synthetic genes** encoding fused proteins
 under tight control of modified regulatory regions of the colicin operon.
 SO GENE, (1992 Aug 1) 117 (1) 7-14.
 Journal code: 7706761. ISSN: 0378-1119.
 AU Waleh N S; Soheli I; Lazar J B; Hudson D V; Sze P; Underhill P A; Johnson P
 H
 AN 92354939 MEDLINE

L61 ANSWER 78 OF 103 HCAPLUS COPYRIGHT 2003 ACS
 TI **High level expression** of basic fibroblast growth
 factor having a homogeneous N-terminus
 SO PCT Int. Appl., 50 pp.

CODEN: PIXXD2

IN Thompson, Stewart A.; Abraham, Judith A.
AN 1992:1768 HCAPLUS
DN 116:1768

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|---|------|----------|-----------------|----------|
| PI | WO 9114785 | A1 | 19911003 | WO 1991-US2186 | 19910328 |
| | W: AU, CA, JP | | | | |
| | RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE | | | | |
| | US 5143829 | A | 19920901 | US 1990-501206 | 19900329 |
| | AU 9176774 | A1 | 19911021 | AU 1991-76774 | 19910328 |
| | AU 660394 | B2 | 19950622 | | |
| | EP 522080 | A1 | 19930113 | EP 1991-908053 | 19910328 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| | JP 05508075 | T2 | 19931118 | JP 1991-507624 | 19910328 |
| | JP 3176916 | B2 | 20010618 | | |
| | JP 2001169793 | A2 | 20010626 | JP 2000-353649 | 19910328 |

L61 ANSWER 79 OF 103 MEDLINE DUPLICATE 23
TI A new family of sugar-inducible expression vectors for Escherichia coli.
SO PROTEIN ENGINEERING, (1991 Oct) 4 (7) 843-7.
Journal code: 8801484. ISSN: 0269-2139.
AU Cagnon C; Valverde V; Masson J M
AN 92187603 MEDLINE

L61 ANSWER 80 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)
TI OVEREXPRESSION OF THE PHAGE LAMBDA LYSOZYME CLONED IN ESCHERICHIA-COLI -
USE OF A DEGENERATIVE MIXTURE OF SYNTHETIC RIBOSOME BINDING-SITES AND
INCREASE OF THE PROTEIN STABILITY INVIVO
SO PROTEIN ENGINEERING, (1991) Vol. 4, No. 4, pp. 485-492.
AU JESPER S L; SONVEAUX E; FASTREZ J (Reprint); PHANAPOULOS A; DAVISON J
AN 91:287093 SCISEARCH

L61 ANSWER 81 OF 103 HCAPLUS COPYRIGHT 2003 ACS
TI Optimization of the signal-sequence cleavage site for secretion from
Bacillus subtilis of a 34-amino acid fragment of human parathyroid hormone
SO Gene (1991), 102(2), 277-82
CODEN: GENED6; ISSN: 0378-1119
AU Saunders, Charles W.; Pedroni, Julia A.; Monahan, Paula M.
AN 1992:1654 HCAPLUS
DN 116:1654

L61 ANSWER 82 OF 103 MEDLINE DUPLICATE 24
TI The upstream activating sequence for L-leucine gene regulation in
Saccharomyces cerevisiae.
SO NUCLEIC ACIDS RESEARCH, (1990 Jul 11) 18 (13) 3923-31.
Journal code: 0411011. ISSN: 0305-1048.
AU Tu H; Casadaban M J
AN 90326515 MEDLINE

L61 ANSWER 83 OF 103 MEDLINE DUPLICATE 25
TI A novel cis-acting DNA element required for a high level of inducible
expression of the rat P-450c gene.
SO MOLECULAR AND CELLULAR BIOLOGY, (1990 Apr) 10 (4) 1470-5.
Journal code: 8109087. ISSN: 0270-7306.
AU Yanagida A; Sogawa K; Yasumoto K I; Fujii-Kuriyama Y
AN 90205824 MEDLINE

L61 ANSWER 84 OF 103 MEDLINE DUPLICATE 26
TI Regulated expression of heterologous genes in Bacillus subtilis using the
Tn10 encoded tet regulatory elements.
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1990 Sep) 33 (6) 657-63.
Journal code: 8406612. ISSN: 0175-7598.
AU Geissendorfer M; Hillen W

AN 91175051 MEDLINE

L61 ANSWER 85 OF 103 MEDLINE

TI **Mutations** in the structural genes for eukaryotic initiation factors 2 alpha and 2 beta of *Saccharomyces cerevisiae* disrupt translational control of GCN4 mRNA.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1989 Oct) 86 (19) 7515-9.
Journal code: 7505876. ISSN: 0027-8424.

AU Williams N P; Hinnebusch A G; Donahue T F

AN 90017508 MEDLINE

L61 ANSWER 86 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

TI Heterologous expression of the alpha-amylase-inhibitor gene cloned from an amplified genomic sequence of *Streptomyces tendae*; tendamistat gene cloning in *Escherichia coli* and *Streptomyces lividans*; gene amplification; DNA sequence

SO J.Bacteriol.; (1989) 171, 9, 4953-57
CODEN: JOBAAY

AU Koller K P; Riess G

AN 1989-12489 BIOTECHDS

L61 ANSWER 87 OF 103 MEDLINE DUPLICATE 27

TI Detection and characterization of the fibroblast growth factor-related oncoprotein INT-2.

SO MOLECULAR AND CELLULAR BIOLOGY, (1989 Nov) 9 (11) 4896-902.
Journal code: 8109087. ISSN: 0270-7306.

AU Dixon M; Deed R; Acland P; Moore R; Whyte A; Peters G; Dickson C

AN 90097905 MEDLINE

L61 ANSWER 88 OF 103 MEDLINE DUPLICATE 28

TI **High-level expression** of self-processed HIV-1 protease in *Escherichia coli* using a **synthetic gene**.

SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1989 Jun 30) 161 (3) 1056-63.
Journal code: 0372516. ISSN: 0006-291X.

AU Hostomsky Z; Appelt K; Ogden R C

AN 89302078 MEDLINE

L61 ANSWER 89 OF 103 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. DUPLICATE 29

TI STRUCTURE OF VACCINIA VIRUS LATE **PROMOTERS**.

SO J MOL BIOL, (1989) 210 (4), 771-784.
CODEN: JMOBAK. ISSN: 0022-2836.

AU DAVISON A J; MOSS B

AN 1990:131339 BIOSIS

L61 ANSWER 90 OF 103 MEDLINE

TI Synthesis and sequence-specific proteolysis of a hybrid protein (colicin A::growth hormone releasing factor) produced in *Escherichia coli*.

SO GENE, (1989 Aug 1) 80 (1) 129-36.
Journal code: 7706761. ISSN: 0378-1119.

AU Geli V; Baty D; Knibiehler M; Lloubes R; Pessegue B; Shire D; Lazdunski C

AN 90006772 MEDLINE

L61 ANSWER 91 OF 103 HCAPLUS COPYRIGHT 2003 ACS

TI **Synthetic genes** for human interleukin 1-.alpha., plasmids containing these genes, and **high-level expression** of the genes in bacteria

SO Eur. Pat. Appl., 12 pp.
CODEN: EPXXDW

IN Zurawski, Gerard; Zurawski, Sandra Marvo

AN 1988:449634 HCAPLUS

DN 109:49634

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|-----|--|------|----------|-----------------|----------|
| | ----- | --- | ---- | ----- | ----- |
| PI | EP 259160 | A2 | 19880309 | EP 1987-307781 | 19870903 |
| | EP 259160 | A3 | 19881130 | | |
| | R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| | US 5017692 | A | 19910521 | US 1986-903497 | 19860904 |
| | EP 481536 | A1 | 19920422 | EP 1991-122275 | 19870903 |
| | R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| | JP 63071185 | A2 | 19880331 | JP 1987-221809 | 19870904 |
| L61 | ANSWER 92 OF 103 MEDLINE DUPLICATE 30 | | | | |
| TI | Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in Escherichia coli. | | | | |
| SO | PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1988 Aug) 85 (16) 5879-83. Journal code: 7505876. ISSN: 0027-8424. | | | | |
| AU | Huston J S; Levinson D; Mudgett-Hunter M; Tai M S; Novotny J; Margolies M N; Ridge R J; Brucoleri R E; Haber E; Crea R; + | | | | |
| AN | 88320347 MEDLINE | | | | |
| L61 | ANSWER 93 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI | | | | |
| TI | Frameshifting at rare codons during high level expression of foreign proteins in E. coli; protease-inhibitor SLPI expression in Escherichia coli (conference abstract) | | | | |
| SO | J.Cell.Biochem.; (1988) Suppl.12D, 70 | | | | |
| AU | Eisenberg S P | | | | |
| AN | 1989-01232 BIOTECHDS | | | | |
| L61 | ANSWER 94 OF 103 MEDLINE DUPLICATE 31 | | | | |
| TI | Structure-function studies on bacteriorhodopsin. III. Total synthesis of a gene for bacterio-opsin and its expression in Escherichia coli. | | | | |
| SO | JOURNAL OF BIOLOGICAL CHEMISTRY, (1987 Jul 5) 262 (19) 9264-70. Journal code: 2985121R. ISSN: 0021-9258. | | | | |
| AU | Nassal M; Mogi T; Karnik S S; Khorana H G | | | | |
| AN | 87250575 MEDLINE | | | | |
| L61 | ANSWER 95 OF 103 MEDLINE DUPLICATE 32 | | | | |
| TI | Roles of the TGACT repeat sequence in the yeast TRP5 promoter . | | | | |
| SO | JOURNAL OF BIOLOGICAL CHEMISTRY, (1987 Mar 15) 262 (8) 3609-14. Journal code: 2985121R. ISSN: 0021-9258. | | | | |
| AU | Moye W S; Zalkin H | | | | |
| AN | 87137656 MEDLINE | | | | |
| L61 | ANSWER 96 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI | | | | |
| TI | Chemical synthesis and in vivo hyperexpression of a modular gene coding for Escherichia coli translational initiation factor IF1; vector construction and cloning (conference abstract) | | | | |
| SO | Protein Eng.; (1987) 1, 3, 253 CODEN: PRENE9 | | | | |
| AU | Calogero R A; Pon C L; Gualerzi C O | | | | |
| AN | 1987-11657 BIOTECHDS | | | | |
| L61 | ANSWER 97 OF 103 MEDLINE DUPLICATE 33 | | | | |
| TI | Chemical synthesis and in vivo hyperexpression of a modular gene coding for Escherichia coli translational initiation factor IF1. | | | | |
| SO | MOLECULAR AND GENERAL GENETICS, (1987 Jun) 208 (1-2) 63-9. Journal code: 0125036. ISSN: 0026-8925. | | | | |
| AU | Calogero R A; Pon C L; Gualerzi C O | | | | |
| AN | 87286424 MEDLINE | | | | |
| L61 | ANSWER 98 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI | | | | |
| TI | Expression of murine epidermal growth factor in Escherichia coli; | | | | |

synthetic gene expression and vector construction
(conference abstract)

SO Biol.Chem.Hoppe Seyler; (1986) 367, Suppl., 162
CODEN: BCHSEI
AU Allen G; Henwood C A; Winther M D
AN 1986-11861 BIOTECHDS

L61 ANSWER 99 OF 103 MEDLINE DUPLICATE 34

TI **High-level expression** of a gene encoding the human complement factor C5a in Escherichia coli.

SO GENE, (1986) 43 (1-2) 131-8.

Journal code: 7706761. ISSN: 0378-1119.

AU Mandecki W; Powell B S; Mollison K W; Carter G W; Fox J L

AN 87005941 MEDLINE

L61 ANSWER 100 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

TI The search for the perfect E.coli expression system;
vector development for maximizing gene expression in Escherichia coli
(conference abstract)

SO Fed.Proc.Fed.Am.Soc.Exp.Biol.; (1985) 44, 4, 1049

CODEN: FEPA7

AU Shatzman A R; Rosenberg M

AN 1985-05769 BIOTECHDS

L61 ANSWER 101 OF 103 MEDLINE DUPLICATE 35

TI Expression of a **synthetic** human growth hormone **gene** in yeast.

SO GENE, (1985) 39 (1) 117-20.

Journal code: 7706761. ISSN: 0378-1119.

AU Tokunaga T; Iwai S; Gomi H; Kodama K; Ohtsuka E; Ikehara M; Chisaka O;
Matsubara K

AN 86083187 MEDLINE

L61 ANSWER 102 OF 103 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 36

TI THE ROLE OF A SIGNALING PROTEIN IN BACTERIAL SENSING BEHAVIORAL EFFECTS OF
INCREASED GENE EXPRESSION.

SO PROC NATL ACAD SCI U S A, (1984) 81 (16), 5056-5060.

CODEN: PNASA6. ISSN: 0027-8424.

AU CLEGG D O; KOSHLAND D E JR

AN 1985:241512 BIOSIS

L61 ANSWER 103 OF 103 HCAPLUS COPYRIGHT 2003 ACS

TI The manufacture and expression of structural genes

SO PCT Int. Appl., 46 pp.

CODEN: PIXXD2

IN Stabinsky, Yitzhak

AN 1984:133585 HCAPLUS

DN 100:133585

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 8304029 | A1 | 19831124 | WO 1983-US563 | 19830415 |
| W: JP | | | | |
| RW: AT, BE, CH, DE, FR, GB, LU, NL, SE | | | | |
| US 4652639 | A | 19870324 | US 1982-375493 | 19820506 |
| EP 108787 | A1 | 19840523 | EP 1983-901773 | 19830415 |
| EP 108787 | B1 | 19900411 | | |
| R: AT, BE, CH, DE, FR, GB, LI, LU, NL, SE | | | | |
| JP 59501096 | T2 | 19840628 | JP 1983-501807 | 19830415 |
| JP 07089934 | B4 | 19951004 | | |
| AT 51873 | E | 19900415 | AT 1983-901773 | 19830415 |
| IL 68491 | A1 | 19900726 | IL 1983-68491 | 19830426 |
| CA 1266628 | A1 | 19900313 | CA 1983-427371 | 19830504 |

=> d ab

2,9,11,13,14,24-27,30,34,35,39,40,44,47,49,50,52,54,61,69,71,74-76,88,91,94,98,101

L61 ANSWER 2 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB DERWENT ABSTRACT:

NOVELTY - Facilitating (M1) production of a protein or its derivative (I) from a negative sense single stranded RNA virus, by expressing a nucleic acid molecule (NAM) encoding (I) in a host cell, where the nucleotide sequence of NAM is optimized for expression by a eukaryotic cell.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an optimized NAM or its derivative, equivalent, analog or mimetic (II); (2) a protein molecule (I) encoded (II); (3) regulating (M2) the functional activity of a viral F protein, where the protein in its non-fully functional form comprises an F2 portion linked, bound or otherwise associated with an F1 portion, and where the F2 portion comprises an intervening peptide sequence, by modulating cleavage of the intervening peptide sequence where excision of a portion of the intervening sequence from the non-fully functional form of the protein up-regulates F protein functional activity; (4) detecting (M3) an agent capable of regulating the functional activity of a viral F protein or its derivative by contacting an eukaryotic cell expressing an optimized NAM with a putative modulatory agent and detecting an altered expression phenotype and/or functional activity; (5) an agent (III) capable of interacting with a viral F protein and modulating a functional activity associated with the viral protein; (6) a viral F protein variant (IV) comprising a **mutation** in the intervening peptide sequence, where the variant exhibits modulated functional activity relative to wild-type F protein or its derivative, homolog, analog, chemical equivalent or mimetic; (7) a recombinant viral construct (RVC) comprising NAM, where the recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to the F protein; and (8) a vaccine comprising RVC.

BIOTECHNOLOGY - Preferred Method: In M1, the virus is from family Paramyxoviridae, and sub-family Pneumovirinae, and more preferably the virus is respiratory syncytial virus (RSV). The protein directly or indirectly facilitates fusion of any one or more viral components with any one or more host cells components, where (I) is a F protein or its derivative, which is the Fsol fragment, or is an N, P or SH protein or its derivative. The eukaryotic host cell is preferably a mammalian cell which is a 293 cell, or a Chinese Hamster Ovary Cell, where the optimization is: (a) a codon optimization which comprises modification of an A and/or T comprising codon to express G and C, respectively and the **splice** site deletion comprises deletion of an RNA **splice** site; and/or (b) a nucleotide **splice** site deletion, where the optimized protein encoding nucleic acid molecule further comprises one or more endonuclease restriction sites, where the optimized F protein encoding nucleic acid sequence corresponds to the sequence defined by a sequence of 1725 ((400)3), 1725 ((400)5), 1575 ((400)4), 1575 ((400)6), 726 ((400)556), 1176 ((400)559), 195 ((400)562), given in the specification, or its derivative. Preferred Method: M2 preferably comprises expressing NAM in a host cell, where the cleavage events occur at the cleavage sites defined by the peptide sequence ((400)564) and ((400)563), and where F protein, in its non-fully functional form, comprises the structure X1X2X3. X1 = non-intervening peptide sequence region of the F2 portion; X2 = the intervening peptide sequence region of the F2 portion; and X3 = F1 portion. The regulation is down regulation. In M3, the viral F protein is a non-fully functional form of the protein and the agent modulates cleavage of the intervening peptide sequence, preferably it modulates the site 2 cleavage event. Preferred Agent: (III) is preferably an antagonist which interacts with a sequence selected from 546 sequences, given in the specification, such as (A) - (E) etc. Preferred Variant: (IV) exhibits down-regulated functional activity relative to wild-type F protein, and comprises a **mutation** in

the cleavage site defined by ((400)564), where the **mutation** comprises amino acid substitution(s) selected from Arg106Gly, Ala107Gln, Arg108Gly, more preferably comprises a sequence of 575 amino acids, given in the specification, or comprises a multiple amino acid deletion from the intervening peptide sequence, where deletion is a partial deletion of the intervening peptide sequence which is (400)569, and where the variant sequence comprises a sequence of 550 ((400)567) amino acids, given in the specification. Preferred Polynucleotide: (II) or its derivative or analog comprises a nucleotide sequence of 3299 or 3450 base pairs, given in the specification. Arg-Ala-Arg-Arg ((400)564) Lys-Lys-Arg-Lys-Arg-Arg ((400)563) Cys-Phe-Ala-Ser-Gly-Gln-Asn-Ile-Thr-Glu (A) Ala-Ser-Gly-Gln-Asn-Ile-Thr-Glu-Glu-Phe (B) Ser-Ala-Val-Ser-Lys-Gly-Tyr-Leu-Ser-Ala (C) Asn-Ala-Val-Thr-Glu-Leu-Gln-Leu-Leu-Met (D) Lys-Lys-Asn-Lys-Cys-Asn-Gly-Thr-Asp-Ala (E) Arg-Ala-Arg-Arg-Glu-Leu-Pro-Arg-Phe-Met-Asn-Tyr-Thr-Leu-Asn-Asn-Ala-Lys-Lys-Thr-Asn-Val-Thr-Leu-Ser (400)569

ACTIVITY - Virucide.

MECHANISM OF ACTION - Vaccine (claimed); Gene therapy. No biological data is given.

USE - (I), especially F protein, is useful for analyzing, designing and/or modifying an agent capable of interacting with a viral F protein or its derivative and modulating a functional activity associated with the protein, by contacting (I) with a putative agent and assessing the degree of interactive complementarity of the agent with the protein (I). An optimized NAM or its derivative, equivalent, analog or mimetic (II), an agent (III) capable of interacting with a viral F protein and modulating a functional activity associated with the viral protein, or an agent identified using (I) is useful in the manufacture of a medicament utilized in the therapeutic and/or prophylactic treatment of conditions characterized by infection with a negative sense single stranded RNA virus, and for modulating a functional activity associated with a viral F protein in a subject, preferably a mammal, especially a human, where the functional activity is F protein mediated host cell virion fusion and/or virion budding and the modulating is down regulation (all claimed).

ADMINISTRATION - Administered respiratorally, intatracheally, nasopharyngeally, intravenously, intraperitoneally, subcutaneously, intracranially, intradermally, intramuscularly, intraocularly, intrathecally, intracerebrally, intranasally, infusion, orally, rectally, or via a drip, patch or implant. Dosage is 0.01 micrograms - 1000 mg/kg.

EXAMPLE - Initial attempts to express the respiratory syncytial virus (RSV) F gene sequence in a soluble form (truncated at the transmembrane domain) proved unsuccessful in achieving **high** levels of **expression**. The sequence used in the expression vectors was called F(sol) (this differed from the viral sequence in 24/1575 nucleotides where restriction sites had been inserted to allow for easy **mutagenesis**). The F viral sequence (F(sol)) contained suboptimal codon usage for expression in mammalian cells. A possible eight 3' **splice** sites were identified, including preceding lariat sequences at four positions. **Poly (A)** adenylation sites (aataaa (400)570) were also identified at 4 positions. The F natural sequence like the viral sequence was approximately 65 % AT rich. Most mammalian expressed genes are less than 50 % AT rich. The DNA sequence encoding the transmembrane form of RSV F. In an attempt to overcome poor expression levels in mammalian cells, a new F sequence was designated that: (i) retained the same encoded amino acid sequence; (ii) used wherever possible optimum codon usage; (iii) removed all potential **splice** sites and **poly A** sites; (iv) removed as many CG doublets as these may be methylation sites; (v) designed unique restriction sites to allow cassette **mutagenesis**; (vi) sequence was checked by secondary structure and any large hairpin loops were destabilized by changing the **sequence**. The **synthetic DNA sequence** Fopt (also referred to as F(sol)) was assembled and cloned. Single stranded synthetic DNA fragments

of average length 60 bases were annealed and ligated together to produce three fragments - a 631 base pair (bp) Pst I-Mfe I fragment, a 606 bp Mfe I-Xho I fragment, and a 379 bp Xho I-Bam HI fragment. These gel purified fragments were cloned in pLitmus 38 or a derivative of pLITMUS (pLITMUS 273/279). Especially fragment Pst-Mfe I, Xho I-Bam HI and Mfe-Xho I were sequentially cloned into the cytomegalovirus (CMV) expression vector pCICO or its derivatives (where pCICO is a derivative of pJW4304 which contains a full length CMV **promoter** and the CMV authentic intron sequence preceding the Pst I site). The 3' terminator used was derived from SV40 early region and this vector also contained the SV40 origin of replication. The plasmid was from the pUC series and contained an ampicillin resistance gene. (pJW4304 was obtained from J. Mullins Dept. of Microbiology, University of Washington, Chapman et al., NAR, 19:3979-3980, 1991). This produced the final clone pCICO.Fopt. pCICO.Fopt was further modified by cloning in a 270 bp EcoRI-Xba I fragment which encodes the transmembrane and cytoplasmic domains of the RSV F protein. (367 pages)

L61 ANSWER 9 OF 103 HCAPLUS COPYRIGHT 2003 ACS

AB The present invention provides **sequences** of a full length and termini truncated **humanized** green fluorescent protein based on Ptilosarcus gurneyi which have been modified to the favored or most favored codons for mammalian expression systems. The disclosed encoded protein has 239 amino acid residues compared with the wild type Ptilosarcus gurneyi which has 238 amino acids. In the present invention, a valine residue has been added at the second position from the amino terminus and **codon preference** bias has been changed in a majority of the wild type codons of Ptilosarcus gurneyi fluorescent protein. The humanized Ptilosarcus gurneyi green fluorescent protein is useful as a fluorescent tag for monitoring the activities of its fusion partners using imaging based approaches.

L61 ANSWER 11 OF 103 HCAPLUS COPYRIGHT 2003 ACS

AB The invention provides modified virus Ankara (MVA), a replication-deficient strain of vaccinia virus, expressing human immunodeficiency virus (HIV) env, gag, and pol genes. The pol gene has reverse transcriptase safety **mutations** in amino acid 185 within the active site of RT, in amino acid 266 which inhibits strand transfer activity, and at amino acid 478 which inhibits the RNaseH activity. In addn., the integrase gene was deleted past EcoRI site. The envelope was truncated in the cytoplasmic tail of the gp41 gene, deleting 115 amino acids of the cytoplasmic tail. The invention demonstrates that a multiprotein DNA/MVA vaccine can raise a memory immune response capable of controlling a highly virulent mucosal immunodeficiency virus challenge. In summary, inventors have made a recombinant MVA virus, MVA/HIV 48, which has **high expression** of the ADA truncated envelope and the HXB2 gag pol. The invention also relates to modified or **synthetic promoters** designed for **gene** expression in MVA or other poxyviruses.

L61 ANSWER 13 OF 103 HCAPLUS COPYRIGHT 2003 ACS

AB A procedure for obtaining curd (bovine rennin) by the expression, not of the sequencing of its natural **gene**, but of an artificial **gene**, **synthetic** and optimized following certain rules of use of triplets in DNA, is described. Preferably, this expression is made with filamentous fungi, esp. with GRAS status, and particularly Aspergillus niger, awamori variant. The synthesis of optimized genes for filamentous fungi, carried out here for the first time for chymosin, allows us to obtain **high** levels of **expression**, which means that the procedure is useful for the industrial prodn. of this valuable protein. Chymosin is obtained extra-cellularly, using a plasmid with a fungal secretion signal, thus allowing for its purifn. by the supernatants from the growth of this fungus, for use in the food industry.

NOVELTY - A new isolated nucleic acid (I) representing a **synthetic BAX-gene**, is new.

DETAILED DESCRIPTION - A new isolated nucleic acid (I) representing a **synthetic BAX-gene** comprises:

- (a) a sequence of 579 bp fully defined in the specification;
- (b) a fragment of (a) encoding a functional fragment of a sequence of 192 amino acids fully defined in the specification;
- (c) any one of 8 nucleotide sequences (not defined in the specification);
- (d) a sequence more than 75% identical to (a), (b) or (c);
- (e) any one of the above sequences interrupted by intervening DNA sequences; or
- (f) a complement of (a)-(d).

INDEPENDENT CLAIMS are also included for the following:

- (1) identifying Bax-resistant yeast or fungi;
- (2) identifying, or obtaining and identifying *Candida* spp. sequences that are differentially expressed in a pathway eventually leading to programmed cell death;
- (3) identifying inhibitors or inhibitor sequences of Bax-induced cell death;
- (4) an isolated *Candida* spp. nucleic acid (II) identified by any of the methods above;
- (5) an isolated polypeptide involved in a pathway for programmed cell death of *Candida* spp. and encoded by (II), where the polypeptide comprises:

- (a) any one of 153 amino acid sequences, e.g. 1381, 321, 251, 403, or 428 amino acids, fully defined in the specification, or encoding a functional equivalent, derivative or bioprecursor of the protein;
- (b) a sequence more than 70% similar or identical to (a); or
- (c) a functional fragment of (a)-(b);
- (6) an antibody capable of specifically binding to the polypeptide or to its specific epitope;

- (7) a pharmaceutical composition comprising the antibody;
- (8) an isolated polypeptide (III) for preparing a medicament for treating diseases associated with yeast or fungi, and involved in a pathway eventually leading to programmed cell death of yeast or fungi, comprises:

- (a) any one of 361 amino acid sequences, e.g. 1381, 321, 251, 403, or 428 amino acids, fully defined in the specification, or encoding a functional equivalent, derivative or bioprecursor of the protein;

- (b) a sequence more than 70% similar or identical to (a); or
- (c) a functional fragment of (a)-(b);
- (9) an isolated nucleic acid (IV) for preparing a medicament for treating diseases associated with yeast or fungi, and encoding a polypeptide involved in a pathway eventually leading to programmed cell death of yeast or fungi, comprises:

- (a) a nucleic acid encoding any of the sequences of (III);
- (b) any one of 351 nucleotide sequences, e.g. 896, 1286, 800, 1535, or 3218 bp fully defined in the specification;

- (c) a sequence more than 70% identical to (b);
- (d) a nucleic acid encoding a functional fragment of (a)-(c);
- (e) a complement of (a)-(d); or
- (f) a human homologue of (a)-(e);

- (10) a pharmaceutical or fungicidal composition comprising (III) or its human homologue, or (IV) or its human homologue, an antisense molecule to at least one of (IV), or an antisense molecule to a mammalian homologue of (IV), with a carrier, diluent or excipient;

- (11) a vaccine for immunizing a mammal against yeast or fungal infections or proliferative disorders, or for preventing apoptosis in certain diseases comprising at least one (III) or its human homologue, or at least one (IV) or its human homologue in a pharmaceutical carrier;

- (12) a genetically modified yeast or fungus, or mammalian cell or

non-human organism, where modification results in the over-expression or under-expression of at least one of (IV) or its human homologue, or at least one of (III) or its human homologue, which prevents, delays or sensitizes for apoptosis of the genetically modified yeast or fungus, or genetically modified mammalian cell or non-human organism;

(13) identifying compounds that selectively modulate expression or functionality of polypeptides involved in a pathway eventually leading to programmed cell death of yeast or fungi or in metabolic pathways where the polypeptides are involved;

(14) identifying compounds or polypeptides that bind to or modulate the properties of polypeptides involved in a pathway eventually leading to programmed cell death of yeast or fungi;

(15) identifying compounds interacting with a polypeptide involved in a pathway eventually leading to programmed cell death of yeast and fungi;

(16) identifying polypeptides involved in a pathway eventually leading to programmed cell death;

(17) a compound or polypeptide identified by the methods of (13) - (16);

(18) preparing a pharmaceutical composition for treating diseases associated with yeast or fungi, or proliferative disorders, or for preventing apoptosis in certain diseases;

(19) a pharmaceutical composition comprising the compound or polypeptide of (17), with a carrier;

(20) preventing yeast or fungal infection;

(21) identifying compounds for stimulating or inhibiting apoptosis;

(22) a compound identified by the method of (21);

(23) an antisense molecule comprising a nucleic acid sequence capable of selectively hybridizing to (II) or (IV);

(24) a nucleic acid probe that selectively hybridizes with (II);

(25) a nucleic acid primer that selectively amplifies (II);

(26) expression vectors comprising (I), (II) or a human homologue of (IV); and

(27) host cells transformed, transfected or infected by any of the vectors of (26).

ACTIVITY - Cytostatic; Fungicide; Immunosuppressive; Virucide; Vasotropic.

No biological data is given.

MECHANISM OF ACTION - Vaccine; Gene therapy.

USE - The isolated nucleic acids, polypeptides, pharmaceutical compositions, antisense molecules and antibodies are useful as medicaments or in preparing a medicament for treating, preventing and/or alleviating diseases associated with yeast or fungi or proliferative disorders, such as cancer, or for preventing apoptosis in certain diseases (all claimed). The compounds or polypeptides identified by the methods above, or the genetically modified organism are useful for preparing a medicament for modifying the endogenic flora of humans and other mammals (claimed). The vaccine is useful for immunizing against yeast or fungal infections. Apoptosis-related diseases include autoimmune disease, ischemia, diseases related with viral infections or neurodegenerations.
Dwg.0/13

L61 ANSWER 24 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)DUPLICATE 4

AB A cDNA, encoding the human growth hormone (hGH), was synthesized based on the known 191 amino acid sequence. Its codon usage was optimized for a **high level expression** in *Escherichia coli*. Unique restriction sites were incorporated throughout the gene to facilitate **mutagenesis** in further studies. To minimize an initiation translation problem, a 624-bp cassette that contained a ribosome binding site and a start codon were fused to the hGH-coding sequence that was flanked between the EcoRI and HindIII sites. The whole fragment was synthesized by an overlapped extension of eight long synthetic oligonucleotides. The four-short duplexes of DNA, which were first formed by annealing and filling-in with a Klenow fragment, were assembled to form a complete hGH gene. The hGH was cloned and expressed successfully using a

pET17b plasmid that contained the T7 **promoter**. Recombinant hGH yielded as much as 20% of the total cellular proteins. However, the majority of the protein was in the form of insoluble inclusion bodies. N-terminal amino acid sequencing also showed that the hGH produced in *E. coli* contained formyl-methionine. This study provides a useful model for synthesis of the gene of interest and production of recombinant proteins in *E. coli*.

- L61 ANSWER 25 OF 103 MEDLINE DUPLICATE 5
AB The phytase gene of *Aspergillus niger* NRRL3135 was modified with a deletion of intron and signal coding sequence. Then, according to the **codon preference** of *Pichia pastoris*, modified phyA gene was artificially synthesized and cloned into expression vector of pPICZ alpha A. The recombinant plasmid was transformed into chromosome of *Pichia pastoris* X-33 strain by electroporation. The results of SDS-PAGE and enzymatic kinetic analysis proved that the recombinant phytase was secreted into culture medium with nearly same character of natural phytase. After screening for high level productive yeast strains, a strain named SPAN-III produced recombinant phytase with 165,000 u/mL under the condition of shake cultivation. It will satisfy the demand for industrialized production in some degree.
- L61 ANSWER 26 OF 103 MEDLINE DUPLICATE 6
AB *Corynebacteria* codon usage exhibits an overall GC content of 67%, and a wobble-position GC content of 88%. *Escherichia coli*, on the other hand has an overall GC content of 51%, and a wobble-position GC content of 55%. The high GC content of *Corynebacteria* genes results in an unfavorable **codon preference** for heterologous expression, and can present difficulties for polymerase-based manipulations due to secondary-structure effects. Since these characteristics are due primarily to base composition at the wobble-position, **synthetic genes** can, in principle, be designed to eliminate these problems and retain the wild-type amino acid sequence. Such genes would obviate the need for special additives or bases during in vitro polymerase-based manipulation and mutant host strains containing uncommon tRNA's for heterologous expression. We have evaluated **synthetic genes** with reduced wobble-position G/C content using two variants of the enzyme 2,5-diketo-D-gluconic acid reductase (2,5-DKGR A and B) from *Corynebacterium*. The wild-type genes are refractory to polymerase-based manipulations and exhibit poor heterologous expression in enteric bacteria. The results indicate that a subset of codons for five amino acids (alanine, arginine, glutamate, glycine and valine) contribute the greatest contribution to reduction in G/C content at the wobble-position. Furthermore, changes in codons for two amino acids (leucine and proline) enhance bias for expression in enteric bacteria without affecting the overall G/C content. The **synthetic genes** are readily amplified using polymerase-based methodologies, and exhibit high levels of heterologous expression in *E. coli*.
- L61 ANSWER 27 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB A modified **synthetic DNA sequence** (I) for improved insect control comprising DNA modified from the truncated crystal protein cry9Aa gene of *Bacillus thuringiensis* ssp. *galleria* are claimed. The encoded crystal protein has a disclosed protein sequence (Ia) of 624 amino acid residues or has a sequence alteration, but the same activity as the active N-terminal domain of the Cry9Aa protein. Also claimed are: a DNA construct for cloning and/or transforming prokaryotic or eukaryotic organisms comprising (I); a prokaryotic or eukaryotic host comprising (I); a method for preparing (I) involving selecting a DNA sequence encoding (Ia) and the unique properties of the Cry9Aa protein and differing from other CryI proteins, providing **synthetic DNA sequences** encoding (Ia), which is encoded by the truncated DNA sequence of 1,989 bp obtainable from the native cry9Aa gene having a 3,837 bp sequence by trypsin (EC-3.4.21.4) cleavage, and improving

translation by changing the **codon preference**; and a method for providing higher plants with insect resistance involving incorporating (I) into a DNA construct and incorporating the construct into a plant to give a transgenic plant. (90pp)

L61 ANSWER 30 OF 103 WPIDS (C) 2003 THOMSON DERWENT
AB WO 200011025 A UPAB: 20000419

NOVELTY - Modified **synthetic DNA sequences** (I) for improved insect control comprising **synthetic DNA sequences** modified from the truncated cry9Aa **gene** of *Bacillus thuringiensis* ssp. *galleria* are new.

DETAILED DESCRIPTION - Modified **synthetic DNA sequences** (I) for improved insect control comprise **synthetic DNA sequences** modified from the truncated cry9Aa **gene** of *Bacillus thuringiensis* ssp. *galleria*, encoding an insecticidal protein having an amino acid sequence of 624 residues (Ia) (given in the specification) or alterations having the same properties as the insecticidally active N-terminal domain of the selected Cry 9Aa protein.

INDEPENDENT CLAIMS are also included for the following:

(1) a DNA construct for cloning and/or transforming prokaryotic or eukaryotic organisms, comprising (I);

(2) a prokaryotic or eukaryotic host comprising (I);

(3) a method for preparing (I) comprising:

(a) selecting a DNA sequence encoding (Ia) and the unique properties of the Cry 9Aa protein and differing substantially from other CryI proteins;

(b) providing **synthetic DNA sequences** encoding (Ia) which is encoded by the truncated DNA sequence of 1989 base pairs (II) obtainable from the native cry9Aa gene having a 3837 base pair sequence (III) (both sequences given in the specification) by trypsin cleavage; and

(c) improving the translation of (II) by changing its **codon preference** in selected direction, in order to obtain modifications of (II) still encoding the insecticidal protein (Ia) or alterations having similar insecticidal action as the insecticidal protein encoded by the native cry9Aa gene of *Bacillus thuringiensis* ssp. *galleria*; and

(4) a method for providing higher plants for improved insecticidal control comprising incorporating (I) into a DNA construct to functionally incorporate (I) into a plant genome.

ACTIVITY - Insecticidal.

No biological data.

MECHANISM OF ACTION - The modified **synthetic DNA sequences** (I) encode (Ia) which, in the gut of the insects, binds to specific receptor molecules with consequent formation of ion channels in the epithelium. This action leads to ion efflux and paralysis of the intestinal function, which causes death of the insect.

USE - The modified **synthetic DNA sequences** (I) are useful for:

(i) producing the unique insecticidal protein (Ia) having the same properties as the N-terminal domain of the insecticidal protein encoded by the native cry9Aa gene of *Bacillus thuringiensis* ssp. *galleria*;

(ii) enhancing expression through improved mRNA processing, stability, and/or translation providing improved tolerance against target insects;

(iii) producing transgenic plants capable of expressing an insecticidal protein similar to (Ia) having the same properties as the N-terminal domain of the insecticidal protein encoded by the native cry9Aa gene of *Bacillus thuringiensis* ssp. *galleria*;

(iv) improving insect resistance in higher plants; and

(v) as an implement in resistance management strategies.

Dwg. 0/25

L61 ANSWER 34 OF 103 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AB With the ready availability of genomic and EST sequence, it is valuable to produce lab-ready genes using only sequence information. Additionally, large sets of related clones can be employed to test the significance of SNPs, potential **splice** variants, and deliberately designed **mutations**. The number of individual genes in these Gene Permutation Sets, GPS, can potentially be large, as researchers seek to determine effects of sequence variation using high throughput analysis techniques. Protein engineering, optimization of protein **expression**, and **high** throughput drug screening can also be expanded in scope and power with isogenic GPS's. Presently, full application of **synthetic gene** technology is limited. The conventional methods are error prone, time consuming, and labor intensive. Picoscript has created an automated process for high throughput assembly of GPS. PicoScripts's Genebuilder technology has the power to produce full-length genes and hundreds of gene variants per day, supplied as ready to use clones. The presentation will focus on the speed, precision, and capacity of this novel gene synthesis system.

L61 ANSWER 35 OF 103 MEDLINE DUPLICATE 9

AB The catalytic domain of the xynB (xylanase) gene from the thermophilic bacterium Dictyoglomus thermophilum was reconstructed by PCR to match the **codon preference** of Trichoderma reesei. The 0.6-kb DNA fragment encoding the enzyme was first amplified by primer extension with a mixture of eight overlapping oligonucleotides, followed by PCR with outside primers containing restriction enzyme sites for directional cloning into Escherichia coli and T. reesei vectors. The **synthetic gene** was expressed in both organisms, producing a clearing halo around transformant colonies in plate assay utilizing an overlay of oat speltis xylan. Effective transcription of xyn B in T. reesei was obtained after changing 20 codons.

L61 ANSWER 39 OF 103 HCAPLUS COPYRIGHT 2003 ACS

AB Several expression plasmid based on the TPO cDNA sequences were initially constructed, but not detectable expression was obsd. Then the gene coding N-terminal 1-153 amino acids of TPO mature peptide was chem. synthesized according to the E. coli preferred codons, and the resultant **synthetic** TPO153 **gene** was subcloned into several expression vectors, among which **high level expression** at 40% of total cellular protein was achieved as GST TPO153 fusion protein. To obtain non-fusion expression of TPO in E. coli, a PCR-based **mutagenesis** of the translation initiation region (TIR) was performed, and expression plasmids based on PRP1, **promoter** were constructed, after transformation of E. coli JM 109, the reconstructed **synthetic** TPO153 **gene** was expressed at the level of about 15% of the total cellular proteins. These results indicate that one of the most important factors that affect expression of rh-TPO in E. coli is the efficiency of translation initiation. Moreover, three expression plasmid based on the hybrid **sequences** of the **synthetic** TPO **gene** and the TPO cDNAs were constructed, and the results indicated that the compn. of downstream sequence could also influence the expression of rh-TPO in E. coli to some extent.

L61 ANSWER 40 OF 103 MEDLINE DUPLICATE 10

AB **Synthetic genes** are very useful in genetic and protein engineering. Here we propose a general method for construction of **synthetic genes**. Short oligonucleotides are joined through ligase chain reaction (LCR) in high stringency conditions to make "unit fragments" which are then fused to form a full-length gene sequence by polymerase chain reaction. The procedure is simple and accurate and does not place constraints on sequence and length. In this report, a recombinant leptin gene was synthesized according to the **codon preference** of Escherichia coli. Besides, a substitution of the only Met at position 54 for Leu and an addition of a Met at the N-terminus were introduced in the **synthetic gene**. The

gene was cloned in the pQE-31 expression vector and was expressed in *E. coli*. A large amount of recombinant leptin containing 6 x His tag was produced and purified by Ni-NTA affinity column. Finally, intact leptin-L54 was released after removing the tag by CNBr cleavage at the Met residue.

L61 ANSWER 44 OF 103 HCAPLUS COPYRIGHT 2003 ACS

AB **Synthetic** DNA mols. encoding HIV **genes** and modifications of HIV genes are provided. The codons of the synthetic mols. use codons preferred by the projected host cell. The synthetic mols. may be used as a polynucleotide vaccine which provides effective immunoprophylaxis against HIV infection through neutralizing antibody and cell-mediated immunity. Thus, genes encoding HIV IIIB env and gag proteins or fragments were engineered to contain codons preferred by human cells. The **synthetic gene** was then examd. for the presence of undesirable elements created by these substitutions (e.g., presence of CG pair created by 3rd nucleotide of new codon and 1st nucleotide of following codon, ATTTA sequences, inadvertent creation of intron splice recognition sites, etc.) and the sequence altered accordingly. Vaccine vector V1Jns consisting of a cytomegalovirus immediate early promoter, bovine growth hormone polyadenylation site, and pUC backbone was employed. Rev-independent expression of env was achieved by replacing its native leader peptide with that from the tissue-specific plasminogen activator gene. I.m. injection of such expression vectors in mice and monkeys resulted in generation of significant protective immunity against subsequent viral challenge. In particular, gp160-specific antibodies and primary CTLs were produced in monkeys.

L61 ANSWER 47 OF 103 MEDLINE DUPLICATE 12

AB *Solanum melongena* (eggplant) cv. Picentia and the wild species *Solanum integrifolium* were transformed with both a wild type (wt) and four **mutagenized** versions of *Bacillus thuringiensis* (Bt) gene Bt43 belonging to the cry3 class. The Bt gene was partly modified in its nucleotide sequence by replacing four target regions (W: +1 to +170; X: +592 to +1057; Y: +1203 to +1376; Z: +1376 to +1984) with synthetic fragments obtained by polymerase chain reaction amplification of crude oligonucleotides. The **synthetic Bt genes** were designed to avoid, in their modified regions, sequences such as ATTTA sequence, **polyadenylation** sequences and splicing sites, which might destabilize the messenger RNA. Furthermore, the codon usage was improved for a better expression in the plant system. The amino acid composition was not altered. Four versions of the modified Bt gene were obtained, BtE, BtF, BtH and BtI, with a nucleotide substitution percentage of 8.2, 8.6, 14, and 16%, respectively, in comparison to the wt gene Bt43. Modified versions contained different subsets of substituted regions: BtE-W + Z, BtF - Y + Z, BtH-X + Y + Z, BtI - W + X + Y + Z. In the final modified version (BtI), overall guanine+cytosine was increased from the 34.1% of the wt gene to 45.5%, and most of the destabilizing sequences were eliminated. Transgenic plants obtained with the more modified versions, BtH and BtI, were fully resistant to *Leptinotarsa decemlineata* Say first- and third-instar larvae, while Bt43 wt, BtE and BtF genotypes did not cause mortality and did not affect larval development.

L61 ANSWER 49 OF 103 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.

AB We have designed protein molecules based on an α -helical coiled-coil structure. These proteins can be tailored to complement nutritionally unbalanced seed meals. In particular, these proteins may contain up to 43% mol/mol of the essential amino acid lysine. **Genes** encoding such proteins were constructed using **synthetic** oligonucleotides and the protein stability was tested for in vivo by expression in an *Escherichia coli* model system. A protein containing 31% lysine and 20% methionine (CP 3-5) was expressed in transgenic tobacco seeds utilizing the seed specific bean phaseolin and soybean β -conglycinin **promoters**. Both **promoters**

provided a level of expression in the mature transgenic tobacco seeds which resulted in a significant increase in the total lysine content of the seeds. Several of these transgenic lines were analyzed for three generations to determine the stability of gene expression. Plants transformed with the soybean .beta.-conglycinin **promoter**/CP 3-5 gene consistently **expressed** the **high-lysine** phenotype through three generations. However, **expression** of the **high-lysine** phenotype in plants transformed with the bean phaseolin/CP 3-5 was variable. This is the first report of a significant increase in seed lysine content due to the seed-specific expression of a de novo protein sequence.

L61 ANSWER 50 OF 103 MEDLINE DUPLICATE 13

AB Stable mammalian cell lines harboring a **synthetic** bovine opsin **gene** have been derived from the suspension-adapted HEK293 cell line. The opsin gene is under the control of the immediate-early cytomegalovirus **promoter**/enhancer in an expression vector that also contains a selectable marker (Neo) governed by a relatively weak **promoter**. The cell lines expressing the opsin gene at high levels are selected by growth in the presence of high concentrations of the antibiotic geneticin. Under the conditions used for cell growth in suspension, opsin is produced at saturated culture levels of more than 2 mg/liter. After reconstitution with 11-cis-retinal, rhodopsin is purified to homogeneity in a single step by immunoaffinity column chromatography. Rhodopsin thus prepared (> 90% recovery at concentrations of up to 15 microM) is indistinguishable from rhodopsin purified from bovine rod outer segments by the following criteria: (i) UV/Vis absorption spectra in the dark and after photobleaching and the rate of metarhodopsin II decay, (ii) initial rates of transducin activation, and (iii) the rate of phosphorylation by rhodopsin kinase. Although mammalian cell opsin migrates slower than rod outer segment opsin on SDS/polyacrylamide gels, presumably due to a different N-glycosylation pattern, their mobilities after deglycosylation are identical. This method has enabled the preparation of several site-specific **mutants** of bovine opsin in comparable amounts.

L61 ANSWER 52 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)DUPLICATE 14

AB To achieve the **high** level **expression** of the bovine growth hormone (bGH) gene in E. coli, the 5'-coding region of the bGH cDNA was modified by replacing the natural **sequence** with various **synthetic** DNA fragments resulting in the change of mRNA secondary structure. The expression plasmids have been constructed using the pKK233-2 vector containing the trc **promoter** (pKBJ1-14). All induced cells harboring these plasmids did not produce a detectable level of bGH. However, replacement of the replication origin with the **mutated** ColE1 replicon resulted in a substantial increase of the bGH production to 3.9%, 27.4%, and 10.9% of total cell protein in pUBJ7, pUBJ9, and pUBJ10, respectively. The analysis of transcripts by dot blot hybridization showed that a post-transcriptional control mechanism is a critical factor for the efficient expression of the bGH gene.

L61 ANSWER 54 OF 103 MEDLINE DUPLICATE 16

AB Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (DHFR-TS) is a well-known target for pyrimethamine and cycloguanil. The low amounts of enzyme obtainable from parasites or the currently available heterologous expression systems have thus far hindered studies of this enzyme. The 1912-base pair P. falciparum DHFR-TS gene was designed based on E. coli **codon preference** with unique restriction sites evenly placed throughout the coding sequence. The gene was designed and synthesized as three separated domains: the DHFR domain, the junctional sequence, and the TS domain. Each of these domains contained numerous unique restriction sites to facilitate mutagenesis. The three domains were assembled into a complete DHFR-TS gene which contained 30 unique restriction sites in the coding **sequence**. The

bifunctional DHFR-TS was expressed from the **synthetic gene** as soluble enzyme in *E. coli* about 10-fold more efficiently than from the wild-type **sequence**. The DHFR-TS from the **synthetic gene** had kinetic properties similar to those of the wild-type enzyme and represents a convenient source of protein for further study. The unique restriction sites in the coding sequence permits easy mutagenesis of the gene which should facilitate further understanding of the molecular basis of antifolate resistance in malaria.

L61 ANSWER 61 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)

AB RAP30 and RAP74 are subunits of RAP30/74 (TFIIF, beta gamma), a general initiation and elongation factor for transcription by RNA polymerase II. Methods were previously published for production of human RAP30 and RAP74 in bacterial cells, using a bacteriophage T7 promoter expression system. The vectors described for production of RAP74 were not very efficient and produced significant quantities of RAP74 amino terminal fragments. To improve these vectors, a segment of the human RAP74 cDNA was recoded using a preferred set of codons for translation in *Escherichia coli*. Recoding dramatically improved protein production and suppressed production of amino-terminal fragments. Improved vectors are reported that produce RAP74 with an LEHHHHHH carboxy-terminal extension (RAP74-H-6), for purification on a Ni²⁺-affinity column, and also with the native carboxy terminus (RAP74). Methods for purification of RAP74-H-6 and RAP74 are reported. Using these improved vectors, approximately 30 mg of soluble and active RAP74-H-6 or RAP74 can be produced and purified from 1 liter off. coil culture, representing a 10-fold improvement in protein production. Methods have also been developed for reconstitution of native RAP30/74 complex using recombinant proteins. This complex has indistinguishable activity from human RAP30/74 for accurate transcription in vitro. (C) 1994 Academic Press, Inc.

L61 ANSWER 69 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)

AB The human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein, gp120 (ENV), is required in large quantities for immunological studies and as a potential vaccine component. We have expressed the DNA encoding gp120 in a highly efficient expression system based on the methylotrophic yeast, *Pichia pastoris*. The native gene was found to contain a sequence which resembled a *Saccharomyces cerevisiae* **polyadenylation** consensus and acted as a premature **polyadenylation** site in *P. pastoris*, resulting in the production of truncated mRNA. As full-length mRNA was produced in *S. cerevisiae*, this indicates differences in mRNA 3'-end formation between the two yeasts. Inactivation of this site by site-directed **mutagenesis** revealed several additional fortuitous **polyadenylation** sites within the **gene**. We have designed and constructed a 69%-**synthetic gene** with increased G+C content which overcomes this transcriptional problem, giving rise to full-length mRNA. High levels of intracellular, insoluble, unglycosylated ENV were produced [1.25 mg/ml in high-density (2 x 10¹⁰) cells per ml fermentations]. ENV also was secreted from *P. pastoris* using the *S. cerevisiae* a-factor prepro secretion leader and the *S. cerevisiae* invertase signal sequence. However, a high proportion of the secreted product was found to be hyperglycosylated, in contrast to other foreign proteins secreted from *P. pastoris*. There also was substantial proteolytic degradation, but this was minimized by maintaining a low pH on induction. Insoluble, yeast-derived ENV proteins are being considered as vaccine antigens, and the *P. pastoris* system offers an efficient method of production.

L61 ANSWER 71 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

AB Various constructs using native or **synthetic genes** with different **promoter** systems were used to establish the requirements for human hemoglobin expression in *Escherichia coli*. When the strong phage T7 **promoter** was used, beta-chains were **expressed** in high yields with both the native cDNA (up

to 10% of E. coli total protein) and artificial genes without the need for a leader peptide. Alpha-chains could not be expressed alone even with the use of the T7 **promoter**. Co-expression of beta chains proved necessary for the expression of active alpha-chains; the operon was placed downstream of the lac **promoter**. This worked only when **synthetic genes** were used, and led to the formation of a fully assembled tetramer (5-10% of total E. coli protein). Optimal codon usage led to enhanced expression, and was essential to provide a rate of protein synthesis necessary for proper folding and heme insertion. The beta-globin produced in the T7 system and the tetrameric hemoglobin produced from **synthetic genes** contained an additional beta-1 Met residue. 2 **Mutants**, beta-1 Val to Met, and beta-1 Val to Ala, were produced using the T7 system. (50 ref)

L61 ANSWER 74 OF 103 SCISEARCH COPYRIGHT 2003 ISI (R)

AB A gene encoding human basic fibroblast growth factor has been chemically synthesized, cloned and expressed in Escherichia coli as a biologically active protein. The 465 bp gene was assembled by enzymatic ligation of 6 pairs of oligonucleotides and cloned in the expression vector pLCII downstream from the strong P(L) **promoter**. This **promoter** directed the synthesis of a fusion protein between a 31 aminoacids fragment of the lambda phage cII protein and bFGF. A four aminoacid recognition sequence for the site-specific protease fXa was introduced in the plasmid construct and this allowed cleavage of the fusion protein at the boundary between cII and bFGF was purified close to homogeneity using a Heparin-Sepharose column and Mono S cation exchange chromatography. The use of the pLCII expression system resulted in the accumulation of 20 to 25 mg of purified bFGF per l of bacterial culture. The recombinant bFGF was mitogenic for mouse 3T3 fibroblasts and the dose-response curve was similar to the one for native bFGF.

L61 ANSWER 75 OF 103 MEDLINE DUPLICATE 21

AB High levels of active HIV-1 protease (PR) were produced in Escherichia coli, amounting to 8-10% of total cell protein. High production levels were achieved by altering the following parameters: (1) **codon preference** of the coding region, (2) A+T-richness at the 5' end of the coding region, and (3) promoter. To circumvent the toxicity of HIV-1 PR in E. coli, the gene was expressed as a fusion protein with two different proteolytic autocleavage sequences. In both the cases, the fusion protein could be cleaved in vivo to give an active molecule with the native sequence at the N terminus.

L61 ANSWER 76 OF 103 MEDLINE

AB A 1974 bp **synthetic gene** was constructed from chemically synthesized oligonucleotides in order to improve transgenic protein expression of the cryIIIA gene from Bacillus thuringiensis var. tenebrionis in transgenic tobacco. The crystal toxin genes (cry) from B. thuringiensis are difficult to express in plants even when under the control of efficient plant regulatory sequences. We identified and eliminated five classes of sequence found throughout the cryIIIA gene that mimic eukaryotic processing signals and which may be responsible for the low levels of transcription and translation. Furthermore, the GC content of the gene was raised from 36% to 49% and the codon usage was changed to be more plant-like. When the **synthetic gene** was placed behind the cauliflower mosaic virus 35S **promoter** and the alfalfa mosaic virus translational enhancer, up to 0.6% of the total protein in transgenic tobacco plants was cryIIIA as measured from immunoblot analysis. Bioassay data using potato beetle larvae confirmed this estimate.

L61 ANSWER 88 OF 103 MEDLINE DUPLICATE 28

AB A **synthetic gene** coding for HIV-1 protease (PR) has been constructed and a system for its efficient expression in E. coli has been established: PR is synthesized as a fusion protein with E. coli

dihydrofolate reductase under the control of a bacteriophage T7 **promoter**. The **synthetic gene** was constructed to enable rapid construction of defined **mutants** by restriction fragment replacement. A set of **mutants** has been constructed which may facilitate elucidation of the mechanism of PR self-cleavage from polyprotein precursors. We have demonstrated that the C-terminal residue (Phe99 in the native sequence) of the processing intermediate is absolutely required for subsequent cleavage at the N-terminal cleavage site. The potential structural role of this residue is discussed with reference to the recently published HIV-1 PR structure.

L61 ANSWER 91 OF 103 HCAPLUS COPYRIGHT 2003 ACS

AB A **synthetic** human interleukin-1.alpha. (IL-1.alpha.) **gene** contg. bacterially preferred codons and 2-5 unique restriction sites is inserted into a vector. The vector may be used to transform bacteria for expression of the IL-1.alpha. gene or **mutants** thereof. A **synthetic** human IL-1.alpha. **gene** contg. a ClaI, an MstI, and a KpnI site was inserted into a plasmid contg. the tac **promoter** and a consensus ribosome binding site. Escherichia coli Transformed with this plasmid produced IL-1.alpha. at 6% of the total protein. Exts. of this transformant contained high levels of IL-1 activity, i.e. 104-106 units/mL (measured by stimulation of IL-2 release from mitogen-stimulated murine lymphoma cell lines).

L61 ANSWER 94 OF 103 MEDLINE DUPLICATE 31

AB We have chemically synthesized a DNA duplex of 757 base pairs which encodes the entire protein sequence of mature bacterio-opsin of Halobacterium halobium. The main aim of the synthesis was to facilitate site-specific **mutagenesis** in all parts of the gene by replacement of short restriction fragments by their counterparts containing the required nucleotide changes. Therefore, 30 unique restriction sites that are fairly evenly spaced were introduced in the synthetic DNA. A total of 28 oligonucleotides ranging in size from 21 to 69 nucleotides were synthesized corresponding to both strands. The entire **gene** was assembled from four **synthetic** fragments of 25, 268, 219, and 245 base pairs. The correctness of the nucleotide sequence was confirmed by sequencing the fragments as well as the complete gene. When expressed under the control of PL **promoter** in Escherichia coli, the **synthetic** and the native **genes** gave similar amounts of bacterio-opsin. Attempts to **increase expression** of the **synthetic gene** by introducing codons that are preferred in E. coli or by introduction of a synthetic transcription terminator were without significant effect.

L61 ANSWER 98 OF 103 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

AB A **synthetic gene** for mouse epidermal growth factor (EGF), a 53-amino acid polypeptide under investigation for de-fleecing sheep, was linked through a codon for lysine to part of the Escherichia coli TrpE gene and a fusion protein was expressed in E.coli under the control of the Trp **promoter**. Biologically active EGF was purified from the fusion protein following lysine-specific proteolysis and a disulfide re-folding step. Alternative sites for cleavage of fusion proteins, including collagenase, Factor-Xa and dilute acid, were investigated, but found ineffective. By site-specific **mutagenesis** a derivative, EGF (Leu 21), was prepared; this was obtained by CNBr digestion of a fusion protein with a linking methionine residue. For **high-level expression** a number of vectors have been investigated including the Tac **promoter** and temperature sensitive runaway-copy-number plasmids. (0 ref)

L61 ANSWER 101 OF 103 MEDLINE DUPLICATE 35

AB A **synthetic** human growth hormone (hGH) **gene** was efficiently expressed under the control of the repressible acid phosphatase promoter in yeast (Saccharomyces cerevisiae). More than 10(6)

molecules of hormone were formed per cell despite the fact that the gene was constructed with **codon preference** for Escherichia coli.

=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

201.37

201.58

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE

TOTAL

ENTRY

SESSION

CA SUBSCRIBER PRICE

-3.91

-3.91

STN INTERNATIONAL LOGOFF AT 18:02:31 ON 31 JAN 2003

| | L # | Hits | Search Text | DBs | Time Stamp |
|----|-----|-------|--|--------------------|---------------------|
| 1 | L1 | 22789 | (humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1) | USPAT; US-PGPUB | 2003/01/31 11:57 |
| 2 | L2 | 2753 | 1 same (regulat\$8 or codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:10 |
| 3 | L3 | 4647 | 1 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 4 | L4 | 1411 | 2 and 3 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 5 | L5 | 966 | 2 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 6 | L6 | 4 | 1 same (regulat\$8 and codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:02 |
| 7 | L7 | 144 | (transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference | USPAT; US-PGPUB | 2003/01/31 12:13 |
| 8 | L8 | 116 | 7 and 1 | USPAT; US-PGPUB | 2003/01/31 14:41 |
| 9 | L9 | 208 | 1 same (luciferase\$1 or gfp) | USPAT; US-PGPUB | 2003/01/31 14:42 |
| 10 | L10 | 30 | 4 and 9 | USPAT; US-PGPUB | 2003/01/31 14:42 |

| | L # | Hits | Search Text | DBs | Time Stamp |
|---|-----|-------|---|--------------------|---------------------|
| 1 | L1 | 22789 | (humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1) | USPAT; US-PGPUB | 2003/01/31 11:57 |
| 2 | L2 | 2753 | 1 same (regulat\$8 or codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:02 |
| 3 | L3 | 4647 | 1 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 4 | L4 | 1411 | 2 and 3 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 5 | L5 | 966 | 2 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 6 | L6 | 4 | 1 same (regulat\$8 and codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:02 |

PGPUB-DOCUMENT-NUMBER: 20020197669

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DOCUMENT-IDENTIFIER: US 20020197669 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------|-------|---------|---------|
| Bangur, Chaitanya S. | Seattle | WA | US | |
| Fanger, Gary Richard | Mill Creek | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| Switzer, Ann P. | Seattle | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Clapper, Jonathan D. | Seattle | WA | US | |

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child 09849626 A1 20010503 parent continuation-in-part-of 09736457 20001213 US
PENDING

US-CL-CURRENT: 435/69.1,435/183 ,435/320.1 ,435/325 ,435/6 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No. 09/736,457, filed Dec. 13, 2000; U.S. patent application Ser. No. 09/702,705, filed Oct. 30, 2000; U.S. patent application Ser. No. 09/677,419, filed Oct. 6, 2000; U.S. patent application Ser. No. 09/671,325, filed Sep. 26, 2000; U.S. patent application Ser. No. 09/658,824, filed Sep. 8, 2000; U.S. patent application Ser. No. 09/651,563, filed Aug. 29, 2000; U.S. patent application Ser. No. 09/614,124, filed Jul. 11, 2000; U.S.

patent application Ser. No. 09/589,184, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000; U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000; U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000; U.S. patent application Ser. No. 09/519,642, filed Mar. 6, 2000; U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999; U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999; U.S. patent application Ser. No. 09/419,356, filed Oct. 15, 1999; U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999; each a CIP of the previous application and all pending.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[1087] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX:

[1288] Tetraspanins are associated with cancer and may play a direct role in controlling tumor progression. Although CD9 expression will positively influence B cell migration, CD9 overexpression suppresses motility and metastasis in carcinoma cells and there is an inverse correlation with metastasis in melanoma. However, CD9 is also expressed on 90% of non-T cell acute lymphoblastic leukemia cells and 50% of chronic lymphocytic leukemias. A recent study using RT-PCR analysis of tetraspanin expression in Burkitt lymphoma cell lines found that 90% of the lines express CD53, CD81, CD63, CD82 and SAS at high levels. CD151/PETA3 is an effector of metastasis and cell migration and Mabs that block this activity have been developed. Similarly, overexpression of the tetraspanin CO-029/D6.1 will increase the metastatic potential of cell lines. The tetraspanins control a diverse set of biological functions that can be **regulated** by Mabs. The functions of the tetraspanins, in general, can be grouped into actions that affect cell activation and proliferation, as well as adhesion and motility. These functions tend to be carried out by their association with integrins. The functional activity of tetraspanins can be modulated with Mabs in such a way as to control cell proliferation. For example, CD81/TAPA-1 is associated with B cell activation and increased proliferation, an activity that can be blocked with Mabs. Mabs with anti-proliferative activity have been generated to the tetraspanin family member CO-029/D6.1. Thus, by comparison, features that make Her2 and CD20 effective therapeutic Mab targets (i.e. control of proliferation) may make

tetraspanins good targets for this type of therapeutic intervention. As mentioned above, L985P is specifically over-expressed in small cell lung carcinoma. Thus, L985P represents an attractive target for therapeutic antibody intervention in small cell lung carcinoma. To facilitate the generation, purification, and evaluation of Mab against L985P, Mabs against the entire deduced amino acid **sequence of the L985P protein, peptides derived from L985P or chemically produced (synthetic)** L985P peptides will be used. Also, one can use Mabs raised against chimeric forms of L985P protein molecule fused to Ra12 protein either the long form (Ra12- which is the first 128 amino acids of Ra12) and/or the short form (Ra12S) or fused to a polyhistidine peptide or any combination of these molecules. Provided are the predicted cDNA and amino acid sequences for the his-tagged L985P-Ra12 fusion molecules: Ra12-L985P_cDNA (SEQ ID NO:1875), Ra12-L985P_Protein (SEQ ID NO:1876), Ra12S-L985P_cDNA (SEQ ID NO: 1877) and Ra12S-L985P_Protein (SEQ ID NO: 1878); and the L985P derived peptides: his-tagged Ra12S-L985PEx_cDNA (SEQ ID NO:1879), his-tagged Ra12S-L985PEx_Protein (SEQ ID NO:1880), L985P_Extracellular_Loop-2_cDNA (SEQ ID NO:1881) and L985P_Extracellular_Loop-2 Peptide (SEQ ID NO:1882).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020187523 A1

TITLE: Extracellular signaling molecules

PUBLICATION-DATE: December 12, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|---------------|-------|---------|---------|
| Tang, Y. Tom | San Jose | CA | US | |
| Yue, Henry | Sunnyvale | CA | US | |
| Lal, Preeti | Santa Clara | CA | US | |
| Burford, Neil | Durham | CT | US | |
| Bandman, Olga | Mountain View | CA | US | |
| Baughn, Mariah R. | San Leandro | CA | US | |
| Azimzai, Yalda | Castro Valley | CA | US | |
| Lu, Dyung Aina M. | San Jose | CA | US | |
| Arvizu, Chandra | Menlo Park | CA | US | |

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| US | PCT/US00/13975 | 2000US-PCT/US00/13975 | May 19, 2000 |

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,800/8

ABSTRACT:

The invention provides human extracellular signaling molecules (EXCS) and polynucleotides which identify and encode EXCS. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of EXCS.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0030] Recently, a unique cytokine has been characterized with a likely role in **regulating** fibrogenesis associated with cases of chronic inflammation. This cytokine, fibrosin, has no obvious homology with other proteins in the GenBank database. A 36-amino acid **synthetic peptide constructed from the deduced amino acid sequence** of human fibrosin stimulates fibroblast growth at subnanomolar concentrations. Tissue fibrosis is a serious complication that accompanies chronic inflammation. A number of fibrogenic cytokines act in concert to stimulate the growth of fibroblasts and the extracellular matrix components associated with fibrosis. (Prakash, S. and P. W. Robbins (1998) DNA Cell Bio. 17:879-884).

Detail Description Paragraph - DETX:

[0150] The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter EXCS-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020061517

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020061517 A1

TITLE: Adenovirus carrying gag gene HIV vaccine

PUBLICATION-DATE: May 23, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|-----------------|-------|---------|---------|
| Chen, Ling | Blue Bell | PA | US | |
| Shiver, John W. | Doylestown | PA | US | |
| Bett, Andrew J. | Lansdale | PA | US | |
| Casimiro, Danilo R. | Harleysville | PA | US | |
| Caulfield, Michael J. | Fort Washington | PA | US | |
| Chastain, Michael A. | Glenside | PA | US | |
| Emini, Emilio A. | Strafford | PA | US | |

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RELATED-US-APPL-DATA:

child 09818443 A1 20010327 parent continuation-in-part-of PCT/US00/18332
20000703 US UNKNOWN non-provisional-of-provisional 60148981 19990813 US
non-provisional-of-provisional 60142631 19990706 US

US-CL-CURRENT: 435/5,424/188.1 ,424/208.1 ,424/233.1 ,435/235.1 ,435/320.1
,435/325

ABSTRACT:

An adenoviral vector is described which carries a codon-optimized gag gene, along with a heterologous promoter and transcription terminator. This viral vaccine can effectively prevent HIV infection when administered to humans either alone or as part of a prime and boost regime also with a vaccine plasmid.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of PCT International Application No. PCT/US00/18332, filed Jul. 3, 2000, which designates the U.S., which claims the benefit, under 35 U.S.C. .sctn. 119(e), of U.S. Provisional Application Ser. No. 60/148,981, filed Aug. 13, 1999 and U.S. Provisional Application Ser. No. 60/142,631, filed Jul. 6, 1999.

----- KWIC -----

Detail Description Paragraph - DETX:

[0050] The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector which specifically includes a gag gene which is codon optimized for expression in a human cellular environment.

Detail Description Paragraph - DETX:

[0092] The full-length (FL) humanized gag gene was ligated into an adenovirus-5 shuttle vector, pHCMVIBGHpA1, containing AdS sequences from bp 1 to bp 341 and bp 3534 to bp 5798 with a expression cassette containing human CMV promoter plus intron A and bovine growth hormone polyadenylation signal. The orientation was confirmed by restriction enzyme digestion analysis and DNA sequencing. Homologous recombination in E. coli was employed using the shuttle plasmid, pA1-CMVI-FLHIV gag, and adenoviral backbone plasmid, pAdE1-E3-, to generate a plasmid form of the recombinant adenovirus containing the expression regulatory elements and FL gag gene, pAd.CMVI-FHIV gag. Appropriate plasmid recombinants were confirmed by restriction enzyme digestion.

US-PAT-NO: 6096304

DOCUMENT-IDENTIFIER: US 6096304 A

TITLE: Recombinant baculovirus insecticides

DATE-ISSUED: August 1, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|------------|-------|----------|---------|
| McCutchen; Billy Fred | Wilmington | DE | N/A | N/A |

APPL-NO: 08/ 952383

DATE FILED: November 13, 1997

PARENT-CASE:

This application is a 371 application of PCT/US96/06988, filed May 16, 1996, which is a continuation in part of Ser. No. 08/443,294, filed May 17, 1995, now abandoned.

PCT-DATA:

APPL-NO: PCT/US96/06988
DATE-FILED: May 16, 1996
PUB-NO: WO96/36712
PUB-DATE: Nov 21, 1996
371-DATE: Nov 13, 1997
102(E)-DATE: Nov 13, 1997

US-CL-CURRENT: 424/93.2; 424/93.6 ; 435/320.1 ; 536/23.4 ; 536/23.5 ; 536/24.1

ABSTRACT:

This invention pertains to recombinant baculoviruses that have been engineered to afford optimal expression of genes encoding insect-selective neurotoxins. More specifically, this invention pertains to an isolated nucleic acid sequence encoding the scorpion toxin LqhIT2, derived from *Leiurus quinquestriatus hebraeus*, wherein the sequence has been optimized for gene expression in nuclear polyhedrosis virus-infected cells. This invention also pertains to chimeric genes comprising a codon-optimized LqhIT2 nucleotide sequence, insecticidal compositions comprising recombinant baculoviruses expressing a codon-optimized, insect-selective neurotoxin (e.g. the LqhIT2 toxin gene), and methods for controlling insects in both agronomic and non-agronomic environments comprising application of insect baculoviruses containing the codon-optimized nucleic acid sequence encoding an insect-selective neurotoxin such as the LqhIT2 toxin.

12 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

----- KWIC -----

Detailed Description Text - DETX:

Many organisms display a bias for use of particular codons to code for insertion of a particular amino acid in a growing peptide chain. **Codon preference** or codon bias, differences in codon usage between organisms, is afforded by degeneracy of the genetic code, and is well documented among many organisms (38-43). Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, inter alia, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in baculovirus expression systems based on optimization of these translational factors. **Synthetic genes** can thus be constructed in order to take advantage of the codon bias displayed by the virus genome. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the virus or the cells in which the virus replicates. Determination of preferred codons can be based on an exhaustive survey of viral and insect genes where sequence information is available. However, this information is of questionable value where the sequence information is derived from insects that are only distantly evolutionarily related to the natural host for the virus. For example, the use of codons biased on the genome of *Drosophila melanogaster* for expression of heterologous genes in AcNPV is not rational since the last common ancestor of flies (e.g., *Drosophila*) and moths (e.g., *Lepidoptera*) dates back approximately 250 million years (44, 45). More useful and compelling information for basing codon selection in favor of gene expression in NPVs can be obtained by simply examining codon bias in genes from the natural hosts of the virus (i.e., lepidopteran insects) and genes encoding highly expressed viral proteins.

Claims Text - CLTX:

5. A chimeric **gene comprising the synthetic gene** of claim 2 operably linked to one or more **regulatory** sequences that direct expression of the coding sequences of the chimeric gene in an insect cell.

| | L # | Hits | Search Text | DBs | Time Stamp |
|---|-----|-------|--|--------------------|---------------------|
| 1 | L1 | 22789 | (humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1) | USPAT; US-PGPUB | 2003/01/31 11:57 |
| 2 | L2 | 2753 | 1 same (regulat\$8 or codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:10 |
| 3 | L3 | 4647 | 1 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 4 | L4 | 1411 | 2 and 3 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 5 | L5 | 966 | 2 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 6 | L6 | 4 | 1 same (regulat\$8 and codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:02 |
| 7 | L7 | 144 | (transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference | USPAT; US-PGPUB | 2003/01/31 12:13 |
| 8 | L8 | 116 | 7 and 1 | USPAT; US-PGPUB | 2003/01/31 13:35 |

PGPUB-DOCUMENT-NUMBER: 20030023036

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030023036 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|---------|-------|---------|---------|
| Houghton, Raymond L. | Bothell | WA | US | |
| Sleath, Paul R. | Seattle | WA | US | |
| Persing, David H. | Redmond | WA | US | |

APPL-NO: 10/ 076622

DATE FILED: February 13, 2002

RELATED-US-APPL-DATA:

child 10076622 A1 20020213 parent continuation-in-part-of 10007805 20011207 US
PENDING child 10007805 20011207 US parent continuation-in-part-of 09834759
20010413 US PENDING child 09834759 20010413 US parent continuation-in-part-of
09620405 20000720 US PENDING child 09620405 20000720 US parent
continuation-in-part-of 09604287 20000622 US PENDING child 09604287 20000622 US
parent continuation-in-part-of 09590751 20000608 US PENDING child 09590751
20000608 US parent continuation-in-part-of 09551621 20000417 US PENDING child
09551621 20000417 US parent continuation-in-part-of 09433826 19991103 US
PENDING child 09433826 19991103 US parent continuation-in-part-of 09389681
19990902 US PENDING child 09389681 19990902 US parent continuation-in-part-of
09339338 19990623 US PENDING child 09339338 19990623 US parent
continuation-in-part-of 09285480 19990402 US PENDING child 09285480 19990402 US
parent continuation-in-part-of 09222575 19981228 US GRANTED parent-patent
6387697 US

US-CL-CURRENT: 530/350

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

----- KWIC -----

Detail Description Paragraph - DETX:

[0729] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX:

[0948] Thirty-one overlapping **synthetic peptides spanning the entire B726P downstream ORF sequence** (amino acid sequence set forth in SEQ ID NO:176) were synthesized and 30 of these were tested in ELISA with sera from breast cancer patients as well as control sera. The amino acid sequences of the 31 overlapping peptides of the B726P downstream ORF are set forth in SEQ ID NO:594-624. Three additional peptides of B726P, set forth in SEQ ID NO:625-627 were also tested. Several peptides throughout the molecule showed reactivity, in particular peptide #2735 (amino acids 31-50; SEQ ID NO:597), peptide #2747 (amino acids 151-170; SEQ ID NO:609), peptide #2750 (amino acids 181-200; SEQ ID NO:612), peptide #2753 (amino acids 211-230; SEQ ID NO:615), and peptide #2766 (amino acids 231-250; SEQ ID NO:617). A total of 16/74 breast cancer sera were reactive with at least one peptide.

PGPUB-DOCUMENT-NUMBER: 20030022254

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030022254 A1

TITLE: Transcription factor regulatory protein

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|---------------|-------|---------|---------|
| Kaser, Matthew R. | Castro Valley | CA | US | |
| Baughn, Mariah R. | San Leandro | CA | US | |

APPL-NO: 10/ 235522

DATE FILED: September 4, 2002

RELATED-US-APPL-DATA:

child 10235522 A1 20020904 parent division-of 09836941 20010417 US GRANTED
parent-patent 6465200 US child 09836941 20010417 US parent division-of 09286132
19990401 US GRANTED parent-patent 6242185 US

US-CL-CURRENT: 435/7.21,435/183 ,435/320.1 ,435/325 ,435/326 ,435/69.1
,435/70.21

ABSTRACT:

The invention provides a mammalian nucleic acid sequence and fragments thereof. It also provides for the use of these nucleic acid sequences in a model system for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with expression of the mammalian nucleic acid sequence. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid sequence.

[0001] This application is a divisional application of U.S. application Ser. No. 09/836,941, filed Apr. 17, 2001, which is a divisional application of U.S. application Ser. No. 09/286,132, filed Apr. 1, 1999, now U.S. Pat. No. 6,242,185, issued Jun. 5, 2001, all of which applications and patents are hereby incorporated by reference herein.

----- KWIC -----

Detail Description Paragraph - DETX:

[0030] "Polypeptide" refers to an amino acid, amino acid **sequence, oligopeptide, peptide, or protein or portions thereof whether naturally occurring or synthetic.**

Detail Description Paragraph - DETX:

[0051] A multitude of polynucleotide sequences capable of encoding the mammalian protein may be cloned into a vector and used to express the protein, or portions thereof, in appropriate host cells. The nucleotide sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change **codon preference** to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain appropriate transcriptional and translational control elements (**promoters**, enhancers, specific initiation signals, and 3' untranslated regions) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid sequence, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

PGPUB-DOCUMENT-NUMBER: 20030018007

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030018007 A1

TITLE: Compositions and methods for inducing gene expression

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|-----------|-------|---------|---------|
| Gregory, Richard J. | Westford | MA | US | |
| Vincent, Karen | Arlington | MA | US | |

APPL-NO: 10/ 190394

DATE FILED: July 3, 2002

RELATED-US-APPL-DATA:

child 10190394 A1 20020703 parent continuation-of 09579897 20000526 US GRANTED
parent-patent 6432927 US child 09579897 20000526 US parent
continuation-in-part-of 09133612 19980813 US ABANDONED child 09133612 19980813
US parent continuation-of PCT/US98/25753 19981204 US PENDING
non-provisional-of-provisional 60067546 19971204 US

US-CL-CURRENT: 514/44,435/320.1 ,435/325 ,435/69.1 ,530/350 ,530/358 ,536/23.2

ABSTRACT:

The present invention provides recombinant nucleic acid molecules encoding a chimeric transactivator protein including a DNA binding domain of a DNA binding protein and a protein domain capable of transcriptional activation. The present invention also provides recombinant viral and non-viral vectors that are able to infect and/or transfect and sustain expression of a biologically active chimeric transactivator proteins in mammalian cells. Also provided are host cell lines and non-human transgenic animals capable of expressing biologically active chimeric transactivator proteins. In another aspect, compositions and methods for treating or preventing ischemic damage associated with hypoxia-related disorders are provided.

[0001] This application claims priority to PCT patent application PCT/US98/25753, filed Dec. 4, 1998, which claims priority to U.S. Ser. No. 09/133,612, filed Aug. 13, 1998, which claims priority to provisional application No. 60/067,546, filed Dec. 4, 1997.

----- KWIC -----

Detail Description Paragraph - DETX:

[0064] The present invention provides novel hybrid/chimeric transactivating proteins comprising a functional portion of a DNA binding protein and a functional portion of a transcriptional activator protein. The hybrid/chimeric transactivating proteins of the invention offer a variety of advantages, including the specific activation of expression of hypoxia-inducible genes containing hypoxia responsive elements (HREs), thereby achieving exceptionally high levels of gene expression. Invention hybrid/chimeric transactivating proteins are capable of functioning in vertebrate cells and may include naturally occurring transcriptional transactivating proteins or domains of proteins from eukaryotic cells including vertebrate cells, viral transactivating proteins or any **synthetic amino acid sequence** that is able to stimulate transcription from a vertebrate promoter. Examples of such transactivating proteins include, but are not limited to, the lymphoid specific transcription factor identified by Muller et al. (Nature 336:544-551 (1988)), the fos protein (Lucibello et al., Oncogene 3:43-52 (1988)); v-jun protein (Bos et al., Cell 52:705-712 (1988)); factor EF-C (Ostapchuk et al., Mol. Cell. Biol. 9:2787-2797 (1989)); HIV-1 tat protein (Arya et al., Science 229:69-73 (1985)), the papillomavirus E2 protein (Lambert et al., J. Virol. 63:3151-3154 (1989)) the adenovirus E1A protein (reviewed in Flint and Shenk, Ann. Rev. Genet. (1989), heat shock factors (HSF1 and HSF2) (Rabindran, et al., PNAS 88:6906-6910 (1991)); the p53 protein (Levine, Cell 88:323-331 (1997), Ko and Prives, Genes Dev. 10:1054-1072 (1996)); Sp1 (Kadonaga, et al. Cell 51:1079-1090 (1987)); AP1 (Lee, et al., Nature 325:368-372 (1987)); CTF/NF1 (Mermoud, et al., Cell 58: 741-753 (1989)), E2F1 (Neuman, et al., Gene 173: 163-169 (1996)); HAP1 (Pfeifer, et al., Cell 56: 291-301 (1989)); HAP2 (Pinkham, et al., Mol.Cell.Biol. 7:578-585 (1987)); MCM1 (Passmore, et al., J. Mol. Biol. 204:593-606 (1988); PHO2 (Sengstag, and Hinnen, NAR 15:233-246 (1987)); and GAL11 (Suzuki et al., Mol. Cell. Biol. 8:4991-4999 (1988)). In preferred embodiments of the invention, the transactivating protein is Herpes simplex virus VP16 (Sadowski et al., Nature 335:563-564 (1988); Triezenberg et al., Genes and Dev. 2:718-729 (1988)), NF.kappa.B ((Schmitz and Baeuerle, EMBO J. 10:3805-3817 (1991); Schmitz, et al., J.Biol.Chem. 269:25613-25620 (1994); and Schmitz, et al., J. Biol. Chem. 270:15576-15584 (1995)), and yeast activators GAL4 and GCN4.

Detail Description Paragraph - DETX:

[0065] Of course, the skilled artisan will understand that transcriptional activation domains useful in the compositions and methods of this invention may also be **synthetic, i.e., based on a sequence** that is not contained within a known, naturally occurring protein. See, for example, Pollock and Gilman, PNAS 94:13388-13389 (1997), which teaches that transcriptional activation is an inherently flexible process in which there is little, if any, requirement for specific structures or stereospecific protein contacts. It also reviews the variety of different molecules that can function as transcriptional activators, including short peptide motifs (as small as eight amino acids), simple amphipathic helices and even mutagenized domains of proteins unrelated to transcriptional activation.

Detail Description Paragraph - DETX:

[0073] Polynucleotides/transgenes are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

Detail Description Paragraph - DETX:

[0074] The skilled artisan will recognize that when expression from the vector is desired, the polynucleotides/transgenes are operatively linked to expression control sequences. Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the human HIF-1.alpha., EPAS1 or HLF polypeptide in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

PGPUB-DOCUMENT-NUMBER: 20030017167

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030017167 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------|------|-------|---------|---------|
| Jiang, Yuqiu | Kent | WA | US | |

APPL-NO: 09/ 904456

DATE FILED: July 11, 2001

RELATED-US-APPL-DATA:

child 09904456 A1 20010711 parent continuation-in-part-of 09878722 20010608 US
PENDING non-provisional-of-provisional 60290240 20010510 US
non-provisional-of-provisional 60256571 20001218 US
non-provisional-of-provisional 60210821 20000609 US

US-CL-CURRENT: 424/185.1,435/320.1 ,435/325 ,435/6 ,435/69.1 ,435/7.23 ,514/44
,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/878,722, filed Jun. 8, 2001, now pending, which application is incorporated herein by reference in its entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0396] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site-**directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference,** produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20030013866

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030013866 A1

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

PUBLICATION-DATE: January 16, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|-----------|-------|---------|---------|
| Daggett, Lorrie P. | San Diego | CA | US | |
| Lu, Chin-Chun | San Diego | CA | US | |

APPL-NO: 10/ 038937

DATE FILED: January 4, 2002

RELATED-US-APPL-DATA:

child 10038937 A1 20020104 parent division-of 08935105 19970929 US GRANTED
parent-patent 6376660 US child 08935105 19970929 US parent division-of 08231193
19940420 US GRANTED parent-patent 5849895 US child 08231193 19940420 US parent
continuation-in-part-of 08052449 19930420 US ABANDONED

US-CL-CURRENT: 536/23.5,435/320.1 ,435/325 ,435/69.1 ,530/350

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA

receptor subunits.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now pending.

----- KWIC -----

Detail Description Paragraph - DETX:

[0062] As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detail Description Paragraph - DETX:

[0083] Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA

sequences which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

PGPUB-DOCUMENT-NUMBER: 20030008299

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030008299 A1

TITLE: Compositions and methods for the therapy and diagnosis of ovarian cancer

PUBLICATION-DATE: January 9, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------|----------|-------|---------|---------|
| Algate, Paul A. | Issaquah | WA | US | |

APPL-NO: 10/ 015219

DATE FILED: December 12, 2001

RELATED-US-APPL-DATA:

child 10015219 A1 20011212 parent continuation-in-part-of 09777564 20010205 US
PENDING non-provisional-of-provisional 60180403 20000204 US
non-provisional-of-provisional 60192745 20000328 US

US-CL-CURRENT: 435/6,435/226 ,435/320.1 ,435/325 ,435/69.1 ,435/7.23 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses an ovarian tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Diagnostic methods based on detecting an ovarian tumor protein, or mRNA encoding such a protein, in a sample are also provided.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0062] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and

PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0142] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus, even from this simplistic description of an extremely complex set of reactions, it is obvious that there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020198362

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020198362 A1

TITLE: Compositions and methods for the detection, diagnosis and therapy of hematological malignancies

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|----------|-------|---------|---------|
| Gaiger, Alexander | Seattle | WA | US | |
| Algate, Paul A. | Issaquah | WA | US | |
| Mannion, Jane | Seattle | WA | US | |

APPL-NO: 09/ 796692

DATE FILED: March 1, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60223378 20000807 US
non-provisional-of-provisional 60223416 20000804 US
non-provisional-of-provisional 60222903 20000803 US
non-provisional-of-provisional 60218950 20000714 US
non-provisional-of-provisional 60206201 20000522 US
non-provisional-of-provisional 60202084 20000504 US
non-provisional-of-provisional 60200999 20000501 US
non-provisional-of-provisional 60200303 20000428 US
non-provisional-of-provisional 60200779 20000428 US
non-provisional-of-provisional 60200545 20000427 US
non-provisional-of-provisional 60190479 20000317 US
non-provisional-of-provisional 60186126 20000301 US

US-CL-CURRENT: 530/350,435/320.1 ,435/325 ,435/6 ,435/69.1 ,536/23.1

ABSTRACT:

Disclosed are methods and compositions for the detection, diagnosis, prognosis, and therapy of hematological malignancies, and in particular, human leukemias and lymphomas of the follicular, Hodgkin's and B cell and T cell non-Hodgkin's types. Disclosed are compositions, methods and kits for eliciting immune and T cell responses to specific malignancy-related antigenic polypeptides and antigenic polypeptide fragments thereof in an animal. Also disclosed are compositions and methods for use in the identification of cells and biological samples containing one or more hematological malignancy-related compositions, and methods for the detection and diagnosis of such diseases and affected cell types. Also disclosed are diagnostic and therapeutic kits, as well as methods

for the diagnosis, therapy and/or prevention of a variety of leukemias and lymphomas.

[0001] The present application claims priority to U.S. Provisional Patent Applications Serial No. 60/186,126, filed Mar. 1, 2000; Serial No. 60/190,479, filed Mar. 17, 2000; Serial No. 60/200,545, filed Apr. 27, 2000; Serial No. 60/200,303, filed Apr. 28, 2000; Serial No. 60/200,779, filed Apr. 28, 2000; Serial No. 60/200,999, filed May 1, 2000; Serial No. 60/202,084, filed May 4, 2000; Serial No. 60/206,201, filed May 22, 2000; Serial No. 60/218,950, filed Jul. 14, 2000; Serial No. 60/222,903, filed Aug. 3, 2000; Serial No. 60/223,416, filed Aug. 4, 2000; and Serial No. 60/223,378, filed Aug. 7, 2000; the entire specification, claims and figures of each of which is specifically incorporated herein by reference in its entirety without disclaimer.

----- KWIC -----

Detail Description Paragraph - DETX:

[0248] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX:

[0323] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020197669

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197669 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------|-------|---------|---------|
| Bangur, Chaitanya S. | Seattle | WA | US | |
| Fanger, Gary Richard | Mill Creek | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| Switzer, Ann P. | Seattle | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Clapper, Jonathan D. | Seattle | WA | US | |

APPL-NO: 09/ 849626

DATE FILED: May 3, 2001

RELATED-US-APPL-DATA:

child 09849626 A1 20010503 parent continuation-in-part-of 09736457 20001213 US
PENDING

US-CL-CURRENT: 435/69.1,435/183 ,435/320.1 ,435/325 ,435/6 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No. 09/736,457, filed Dec. 13, 2000; U.S. patent application Ser. No. 09/702,705, filed Oct. 30, 2000; U.S. patent application Ser. No. 09/677,419, filed Oct. 6, 2000; U.S. patent application Ser. No. 09/671,325, filed Sep. 26, 2000; U.S. patent application Ser. No. 09/658,824, filed Sep. 8, 2000; U.S. patent application Ser. No. 09/651,563, filed Aug. 29, 2000; U.S. patent application Ser. No. 09/614,124, filed Jul. 11, 2000; U.S.

patent application Ser. No. 09/589,184, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000; U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000; U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000; U.S. patent application Ser. No. 09/519,642, filed Mar. 6, 2000; U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999; U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999; U.S. patent application Ser. No. 09/419,356, filed Oct. 15, 1999; U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999; each a CIP of the previous application and all pending.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[1087] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX:

[1288] Tetraspanins are associated with cancer and may play a direct role in controlling tumor progression. Although CD9 expression will positively influence B cell migration, CD9 overexpression suppresses motility and metastasis in carcinoma cells and there is an inverse correlation with metastasis in melanoma. However, CD9 is also expressed on 90% of non-T cell acute lymphoblastic leukemia cells and 50% of chronic lymphocytic leukemias. A recent study using RT-PCR analysis of tetraspanin expression in Burkitt lymphoma cell lines found that 90% of the lines express CD53, CD81, CD63, CD82 and SAS at high levels. CD151/PETA3 is an effector of metastasis and cell migration and Mabs that block this activity have been developed. Similarly, overexpression of the tetraspanin CO-029/D6.1 will increase the metastatic potential of cell lines. The tetraspanins control a diverse set of biological functions that can be regulated by Mabs. The functions of the tetraspanins, in general, can be grouped into actions that affect cell activation and proliferation, as well as adhesion and motility. These functions tend to be carried out by their association with integrins. The functional activity of tetraspanins can be modulated with Mabs in such a way as to control cell proliferation. For example, CD81/TAPA-1 is associated with B cell activation and increased proliferation, an activity that can be blocked with Mabs. Mabs with anti-proliferative activity have been generated to the tetraspanin family member CO-029/D6.1. Thus, by comparison, features that make Her2 and CD20 effective therapeutic Mab targets (i.e. control of proliferation) may make

tetraspanins good targets for this type of therapeutic intervention. As mentioned above, L985P is specifically over-expressed in small cell lung carcinoma. Thus, L985P represents an attractive target for therapeutic antibody intervention in small cell lung carcinoma. To facilitate the generation, purification, and evaluation of Mab against L985P, Mabs against the entire deduced amino acid **sequence of the L985P protein, peptides derived from L985P or chemically produced (synthetic)** L985P peptides will be used. Also, one can use Mabs raised against chimeric forms of L985P protein molecule fused to Ra12 protein either the long form (Ra12- which is the first 128 amino acids of Ra12) and/or the short form (Ra12S) or fused to a polyhistidine peptide or any combination of these molecules. Provided are the predicted cDNA and amino acid sequences for the his-tagged L985P-Ra12 fusion molecules: Ra12-L985P_cDNA (SEQ ID NO:1875), Ra12-L985P_Protein (SEQ ID NO:1876), Ra12S-L985P_cDNA (SEQ ID NO: 1877) and Ra12S-L985P_Protein (SEQ ID NO: 1878); and the L985P derived peptides: his-tagged Ra12S-L985PEx_cDNA (SEQ ID NO:1879), his-tagged Ra12S-L985PEx_Protein (SEQ ID NO:1880), L985P_Extracellular_Loop-2_cDNA (SEQ ID NO:1881) and L985P_Extracellular_Loop-2 Peptide (SEQ ID NO:1882).

PGPUB-DOCUMENT-NUMBER: 20020194648

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020194648 A1

TITLE: Plasmodium falciparum merozoite surface protein-1 malaria produced in transgenic plants

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|----------|-------|---------|---------|
| Chang, Sandra P. | Honolulu | HI | US | |
| Christopher, David A. | Honolulu | HI | US | |
| Vine, Benjamin | Honolulu | HI | US | |
| Su, Wei-Wen | Honolulu | HI | US | |
| Bugos, Robert | Honolulu | HI | US | |

APPL-NO: 10/ 098514

DATE FILED: March 11, 2002

RELATED-US-APPL-DATA:

child 10098514 A1 20020311 parent continuation-in-part-of 09500376 20000208 US
PENDING non-provisional-of-provisional 60274599 20010309 US

US-CL-CURRENT: 800/294,435/252.2 ,435/320.1 ,536/23.2 ,800/317.3

ABSTRACT:

This invention is in the field of recombinant Plasmodium falciparum polypeptides and relates to recombinant or synthetic antigen compositions which comprise p42 antigens, and more specifically to methods and compositions for the expression of Plasmodium falciparum polypeptides in transgenic plants.

[0001] This application claims priority to U.S. Provisional Application Serial No. 60/274,599, filed Mar. 9, 2001; and is a continuation-in-part of U.S. patent application Ser. No. 09/500,376, filed Feb. 8, 2000.

----- KWIC -----

Detail Description Paragraph - DETX:

[0177] To eliminate the potential problematic sequences of the MSP1.42 FUP and FVO genes (e.g., Examples 1 and 2), a synthetic FUP MSP1.42 sequence (NtMSP1.42) having a modified codon preference and lower A:T content (56%) was

used to generate another series of constructs. The A:T content of NtMSP1.42 was closer to that of the consensus codon usage for tobacco and the NtMSP1.42 sequence contained no potential poly-adenylation sequences, cryptic intron splice sites and only one ATTTA RNA instability sequence, which was removed prior to tobacco transformation. Two different constructs using NtMSP1.42 were created: the first contained an upstream signal sequence and 3' ER retention signal and was targeted for secretion (NtMSP1.42S) and the second contained only the ER retention signal and thus would be retained in the cytoplasm (NtMSP1.42C). RNA preparations of transgenic plants that contained either NtMSP1.42S or NtMSP1.42C contained hybridizing bands of the expected size for intact MSP1.42 mRNA and, in some cases, additional larger sized RNA species. More NtMSP1.42S than NtMSP1.42C plants contained specific transcripts. Transgenic plants containing both constructs were positive by Western blot for the 42 kD MSP1.42 protein. Expression levels were consistently higher in plants containing the secretory, MSP1.42S construct than in transgenic plants containing the cytoplasmic, NtMSP1.42C construct.

PGPUB-DOCUMENT-NUMBER: 20020193329

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020193329 A1

TITLE: Compositions and methods for the therapy and diagnosis of Her-2/neu-associated malignancies

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|---------------|-------|---------|---------|
| Hand-Zimmermann, Susan | Redmond | WA | US | |
| Cheever, Martin A. | Mercer Island | WA | US | |
| Foy, Teresa M. | Federal Way | WA | US | |
| Lodes, Michael J. | Seattle | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |

APPL-NO: 09/ 930125

DATE FILED: August 14, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60270520 20010221 US
non-provisional-of-provisional 60236428 20000928 US
non-provisional-of-provisional 60225152 20000814 US

US-CL-CURRENT: 514/44,424/185.1 ,435/226 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly Her-2/neu-associated cancers, are disclosed. Illustrative compositions comprise one or more Her-2/neu polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of Her-2/neu-associated malignancies.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/270,520 filed Feb. 21, 2001, U.S. Provisional Application No. 60/236,428 filed Sep. 28, 2000, and U.S. Provisional Application No. 60/225,152 filed Aug. 14, 2000, incorporated in their entirety herein.

----- KWIC -----

Detail Description Paragraph - DETX:

[0149] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference,** produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020193296

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020193296 A1

TITLE: Compositions and methods for the therapy and diagnosis of prostate cancer

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------------|-------------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Stolk, John A. | Bothell | WA | US | |
| Day, Craig H. | Shoreline | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A. W. | Bellevue | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Hural, John | Bainbridge Island | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |
| Bassols, Carlota Vinals | Rixensart | WA | BE | |
| y de | Federal Way | US | | |
| Foy, Teresa M. | | | | |

APPL-NO: 09/ 895814

DATE FILED: June 29, 2001

RELATED-US-APPL-DATA:

child 09895814 A1 20010629 parent continuation-in-part-of 09852911 20010509 US
PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09780669
20010209 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of
09759143 20010112 US PENDING child 09895814 A1 20010629 parent
continuation-in-part-of 09709729 20001109 US ABANDONED child 09895814 A1
20010629 parent continuation-in-part-of 09685166 20001010 US PENDING child
09895814 A1 20010629 parent continuation-in-part-of 09679426 20001002 US
PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09657279

20000906 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09651236 20000829 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09636215 20000809 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09605783 20000627 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09593793 20000613 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09570737 20000512 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09568100 20000509 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09536857 20000327 US ABANDONED child 09895814 A1 20010629 parent continuation-in-part-of 09483672 20000114 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09443686 19991118 US ABANDONED child 09895814 A1 20010629 parent continuation-in-part-of 09439313 19991112 US PATENTED child 09895814 A1 20010629 parent continuation-in-part-of 09352616 19990713 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09288946 19990409 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09232149 19990115 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09159812 19980923 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09115453 19980714 US PENDING child 09895814 A1 20010629 parent continuation-in-part-of 09030607 19980225 US PATENTED child 09895814 A1 20010629 parent continuation-in-part-of 09020956 19980209 US PATENTED child 09895814 A1 20010629 parent continuation-in-part-of 08904804 19970801 US ABANDONED child 09895814 A1 20010629 parent continuation-in-part-of 08806099 19970225 US ABANDONED

US-CL-CURRENT: 514/12,435/183 ,435/320.1 ,435/325 ,435/6 ,435/69.1 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No. 09/852,911, filed May 9, 2001; U.S. patent application Ser. No. 09/780,669, filed Feb. 9, 2001; U.S. patent application Ser. No. 09/759,143, filed Jan. 12, 2001; U.S. patent application Ser. No. 09/709,729, filed Nov. 9, 2000; U.S. patent application Ser. No. 09/685,166, filed Oct. 10, 2000; U.S. patent application Ser. No. 09/679,426, filed Oct. 2, 2000; U.S. patent application Ser. No. 09/657,279, filed Sep. 6, 2000; U.S. application Ser. No. 09/651,236, filed Aug. 29, 2000; U.S. application Ser. No. 09/636,215, filed Aug. 9, 2000; U.S. application Ser. No. 09/605,783, filed Jun. 27, 2000; U.S. application Ser. No. 09/593,793, filed Jun. 13, 2000; U.S. application Ser. No. 09/570,737, filed May 12, 2000; U.S. application Ser. No. 09/568,100, filed May 9, 2000; U.S. application Ser. No. 09/536,857, filed Mar. 27, 2000; U.S. application Ser. No. 09/483,672, filed Jan. 14,

2000; U.S. application Ser. No. 09/443,686, filed Nov. 18, 1999; U.S. application Ser. No. 09/439,313, filed Nov. 12, 1999; U.S. application Ser. No. 09/352,616, filed Jul. 13, 1999; U.S. application Ser. No. 09/288,946, filed Apr. 9, 1999; U.S. application Ser. No. 09/232,149, filed Jan. 15, 1999; U.S. application Ser. No. 09/159,812, filed Sep. 23, 1998; U.S. application Ser. No. 09/115,453, filed Jul. 14, 1998; U.S. application Ser. No. 09/030,607, filed Feb. 25, 1998; U.S. application Ser. No. 09/020,956, filed Feb. 9, 1998; U.S. application Ser. No. 08/904,804, filed Aug. 1, 1997 (abandoned); U.S. application Ser. No. 08/806,099, filed Feb. 25, 1997 (abandoned); each a CIP of the previously filed application and pending unless noted, and all incorporated in their entirety herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX:

[0790] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020192763

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020192763 A1

TITLE: Compositions and methods for the therapy and diagnosis of prostate cancer

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|-------------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Stolk, John A. | Bothell | WA | US | |
| Day, Craig H. | Shoreline | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A. W. | Bellevue | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Hural, John | Bainbridge Island | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |
| y de Bassols, Carlota | Rixensart | WA | BE | |
| Vinals | Federal Way | | US | |
| Foy, Teresa M. | | | | |

APPL-NO: 09/ 895793

DATE FILED: June 29, 2001

RELATED-US-APPL-DATA:

child 09895793 A1 20010629 parent continuation-in-part-of 09822827 20010328 US
PENDING child 09822827 20010328 US parent continuation-in-part-of 09679272
20001004 US PENDING non-provisional-of-provisional 60157455 20000417 US

US-CL-CURRENT: 435/69.7,435/183 ,435/320.1 ,435/325 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

----- KWIC -----

Detail Description Paragraph - DETX:

[0794] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020183499

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020183499 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|---------|-------|---------|---------|
| Lodes, Michael J. | Seattle | WA | US | |
| Mohamath, Raodoh | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Benson, Darin R. | Seattle | WA | US | |
| Secrist, Heather | Seattle | WA | US | |

APPL-NO: 09/ 854133

DATE FILED: May 11, 2001

RELATED-US-APPL-DATA:

child 09854133 A1 20010511 parent continuation-in-part-of 09738973 20001214 US
PENDING child 09738973 20001214 US parent continuation-in-part-of 09704512
20001101 US PENDING child 09704512 20001101 US parent continuation-in-part-of
09667170 20000920 US PENDING child 09667170 20000920 US parent
continuation-in-part-of 09640878 20000818 US PENDING child 09667170 20000920 US
parent continuation-in-part-of 09588937 20000605 US PENDING child 09667170
20000920 US parent continuation-in-part-of 09538037 20000329 US ABANDONED child
09667170 20000920 US parent continuation-in-part-of 09518809 20000303 US
ABANDONED child 09667170 20000920 US parent continuation-in-part-of 09476235
19991230 US ABANDONED child 09667170 20000920 US parent continuation-in-part-of
09370838 19990809 US PENDING child 09667170 20000920 US parent
continuation-in-part-of 09285323 19990402 US ABANDONED

US-CL-CURRENT: 536/23.1,424/184.1 ,435/320.1 ,435/325 ,435/7.23 ,530/324
,536/24.3

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

[0001] CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application is related to U.S. patent application Ser. No. 09/738,973, filed Dec. 14, 2000; U.S. patent application Ser. No. 09/704,512, filed Nov. 1, 2000; U.S. patent application Ser. No. 09/667,170, filed Sep. 20, 2000; U.S. Provisional application Ser. No. 60/229,763, filed Sep. 1, 2000; U.S. patent application Ser. No. 09/640,878, filed Aug. 18, 2000; U.S. patent application Ser. No. 09/588,937, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/538,037, filed Mar. 29, 2000; U.S. patent application Ser. No. 09/518,809, filed Mar. 3, 2000; U.S. patent application Ser. No. 09/476,235 filed Dec. 30, 1999; U.S. patent application Ser. No. 09/370,838, filed Aug. 9, 1999; and U.S. patent application Ser. No. 09/285,323, filed Apr. 2, 1999, each a CIP of the previous application and all pending, and incorporated herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX:

[0806] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020183251

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020183251 A1

TITLE: Compositions and methods for the therapy and diagnosis of prostate cancer

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|-------------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Stolk, John A. | Bothell | WA | US | |
| Day, Craig H. | Shoreline | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A.W. | Bellevue | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Hural, John | Bainbridge Island | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |
| Vinals y de Bassols, | Rixensart | WA | BE | |
| Carlota | Federal Way | WA | US | |
| Foy, Teresa M. | Mercer Island | WA | US | |
| Watanabe, Yoshihiro | Seattle | | US | |
| Meagher, Madeleine Joy | | | | |

APPL-NO: 10/ 012896

DATE FILED: December 10, 2001

RELATED-US-APPL-DATA:

child 10012896 A1 20011210 parent continuation-in-part-of 09895814 20010629 US
PENDING child 09895814 20010629 US parent continuation-in-part-of 09852911
20010509 US PENDING child 09852911 20010509 US parent continuation-in-part-of
09780669 20010209 US PENDING child 09780669 20010209 US parent
continuation-in-part-of 09759143 20010112 US PENDING child 09759143 20010112 US
parent continuation-in-part-of 09709729 20001109 US ABANDONED child 09709729

20001109 US parent continuation-in-part-of 09685166 20001010 US PENDING child 09685166 20001010 US parent continuation-in-part-of 09679426 20001002 US PENDING child 09679426 20001002 US parent continuation-in-part-of 09657279 20000906 US PENDING child 09657279 20000906 US parent continuation-in-part-of 09651236 20000829 US PENDING child 09651236 20000829 US parent continuation-in-part-of 09636215 20000809 US PENDING child 09636215 20000809 US parent continuation-in-part-of 09605783 20000627 US PENDING child 09605783 20000627 US parent continuation-in-part-of 09593793 20000613 US PENDING child 09593793 20000613 US parent continuation-in-part-of 09570737 20000512 US PENDING child 09570737 20000512 US parent continuation-in-part-of 09568100 20000509 US PENDING child 09568100 20000509 US parent continuation-in-part-of 09536857 20000327 US ABANDONED child 09536857 20000327 US parent continuation-in-part-of 09483672 20000114 US PENDING child 09483672 20000114 US parent continuation-in-part-of 09443686 19991118 US ABANDONED child 09443686 19991118 US parent continuation-in-part-of 09439313 19991112 US GRANTED parent-patent 6329505 US child 09439313 19991112 US parent continuation-in-part-of 09352616 19990713 US GRANTED parent-patent 6395278 US child 09352616 19990713 US parent continuation-in-part-of 09288946 19990409 US PENDING child 09288946 19990409 US parent continuation-in-part-of 09232149 19990115 US PENDING child 09232149 19990115 US parent continuation-in-part-of 09159812 19980923 US PENDING child 09159812 19980923 US parent continuation-in-part-of 09115453 19980714 US PENDING child 09115453 19980714 US parent continuation-in-part-of 09030607 19980225 US GRANTED parent-patent 6262245 US child 09030607 19980225 US parent continuation-in-part-of 09020956 19980209 US GRANTED parent-patent 6261562 US child 09020956 19980209 US parent continuation-in-part-of 08904804 19970801 US ABANDONED child 08904804 19970801 US parent continuation-in-part-of 08806099 19970225 US ABANDONED

US-CL-CURRENT: 514/12,435/183 ,435/320.1 ,435/325 ,435/6 ,435/69.1 ,530/350 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No. 09/895,814, filed Jun. 29, 2001; U.S. patent application Ser. No. 09/852,911, filed May 9, 2001; U.S. patent application Ser. No. 09/780,669, filed Feb. 9, 2001; U.S. patent application Ser. No. 09/759,143, filed Jan. 12, 2001; U.S. patent application Ser. No. 09/709,729, filed Nov. 9, 2000; U.S. patent application Ser. No. 09/685,166, filed Oct. 10, 2000; U.S. patent application Ser. No. 09/679,426, filed Oct. 2, 2000; U.S. patent application Ser. No. 09/657,279, filed Sep. 6, 2000; U.S. Application Ser.

No. 09/651,236, filed Aug. 29, 2000; U.S. application Ser. No. 09/636,215, filed Aug. 9, 2000; U.S. application Ser. No. 09/605,783, filed Jun. 27, 2000; U.S. application Ser. No. 09/593,793, filed Jun. 13, 2000; U.S. application Ser. No. 09/570,737, filed May 12, 2000; U.S. application Ser. No. 09/568,100, filed May 9, 2000; U.S. application Ser. No. 09/536,857, filed Mar. 27, 2000; U.S. application Ser. No. 09/483,672, filed Jan. 14, 2000; U.S. application Ser. No. 09/443,686, filed Nov. 18, 1999; U.S. application Ser. No. 09/439,313, filed Nov. 12, 1999; U.S. application Ser. No. 09/352,616, filed July 13, 1999; U.S. application Ser. No. 09/288,946, filed Apr. 9, 1999; U.S. application Ser. No. 09/232,149, filed Jan. 15, 1999; U.S. application Ser. No. 09/159,812, filed Sep. 23, 1998; U.S. application Ser. No. 09/115,453, filed Jul. 14, 1998; U.S. application Ser. No. 09/030,607, filed Feb. 25, 1998; U.S. application Ser. No. 09/020,956, filed Feb. 9, 1998; U.S. application Ser. No. 08/904,804, filed Aug. 1, 1997 (abandoned); U.S. application Ser. No. 08/806,099, filed Feb. 25, 1997 (abandoned); each a CIP of the previously filed application and pending unless noted, and all incorporated in their entirety herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX:

[0803] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020177552

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020177552 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: November 28, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|---------|-------|---------|---------|
| Jiang, Yuqiu | Kent | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Secrist, Heather | Seattle | WA | US | |

APPL-NO: 09/ 878178

DATE FILED: June 8, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60270216 20010220 US
non-provisional-of-provisional 60210899 20000609 US

US-CL-CURRENT: 514/12,435/183 ,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/270,216 filed Feb. 20, 2001 and U.S. Provisional Application No. 60/210,899 filed Jun. 9, 2000, incorporated by reference in their entirety herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0148] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020173638

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020173638 A1

TITLE: Compositions and methods for the therapy and diagnosis of ovarian cancer

PUBLICATION-DATE: November 21, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|------|-------|---------|---------|
| Stolk, John A. | | US | | |
| Molesh, David Alan | | US | | |
| Fling, Steven P. | | US | | |
| Xu, Jiangchun | | US | | |

APPL-NO: 09/ 970966

DATE FILED: October 2, 2001

RELATED-US-APPL-DATA:

child 09970966 A1 20011002 parent continuation-in-part-of 09825294 20010403 US
PENDING child 09825294 20010403 US parent continuation-in-part-of 09713550
20001114 US PENDING child 09713550 20001114 US parent continuation-in-part-of
09656668 20000907 US PENDING child 09656668 20000907 US parent
continuation-in-part-of 09640173 20000815 US PENDING child 09640173 20000815 US
parent continuation-in-part-of 09561778 20000501 US PENDING child 09561778
20000501 US parent continuation-in-part-of 09394374 19990910 US ABANDONED

US-CL-CURRENT: 536/23.2,435/183 ,435/6 ,435/7.23

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/825,294, filed Apr. 3, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/713,550, filed Nov. 14, 2000, which is a

continuation-in-part of Ser. No. 09/656,668, filed Sep. 7, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/640,173, filed Aug. 15, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/561,778, filed May 1, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/394,374, filed Sep. 10, 1999, now abandoned, each of which applications are incorporated by reference in their entirety herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0166] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020172952

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020172952 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: November 21, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|---------------|-------|---------|---------|
| Henderson, Robert A. | Edmonds | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| Watanabe, Yoshihiro | Mercer Island | WA | US | |
| Johnson, Jeffrey C. | Des Moines | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Durham, Margarita | Seattle | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Bangur, Chaitanya S. | Seattle | WA | US | |
| McNabb, Andria | Renton | WA | US | |

APPL-NO: 09/ 902941

DATE FILED: July 10, 2001

RELATED-US-APPL-DATA:

child 09902941 A1 20010710 parent continuation-in-part-of 09849626 20010503 US
PENDING child 09849626 20010503 US parent continuation-in-part-of 09736457
20001213 US PENDING child 09736457 20001213 US parent continuation-in-part-of
09702705 20001030 US PENDING child 09702705 20001030 US parent
continuation-in-part-of 09677419 20001006 US PENDING child 09677419 20001006 US
parent continuation-in-part-of 09671325 20000926 US PENDING child 09671325
20000926 US parent continuation-in-part-of 09658824 20000908 US PENDING child
09658824 20000908 US parent continuation-in-part-of 09651563 20000829 US
PENDING child 09651563 20000829 US parent continuation-in-part-of 09614124
20000711 US PENDING child 09614124 20000711 US parent continuation-in-part-of
09589184 20000605 US PENDING child 09589184 20000605 US parent
continuation-in-part-of 09560406 20000427 US PENDING child 09560406 20000427 US
parent continuation-in-part-of 09546259 20000410 US PENDING child 09546259
20000410 US parent continuation-in-part-of 09533077 20000322 US PENDING child
09533077 20000322 US parent continuation-in-part-of 09519642 20000306 US
PENDING child 09519642 20000306 US parent continuation-in-part-of 09476300
19991230 US PENDING child 09476300 19991230 US parent continuation-in-part-of
09466867 19991217 US PENDING child 09466867 19991217 US parent
continuation-in-part-of 09419356 19991015 US PENDING child 09419356 19991015 US
parent continuation-in-part-of 09346492 19990630 US PENDING

US-CL-CURRENT: 435/6,435/183 ,435/320.1 ,435/325 ,435/69.1 ,435/7.23 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

----- KWIC -----

Detail Description Paragraph - DETX:

[1160] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX:

[1383] To facilitate the generation, purification, and evaluation of MAb against L985P, MAbs against the entire deduced amino acid **sequence of the L985P protein, peptides derived from L985P or chemically produced (synthetic)** L985P peptides will be used. Also, one can use MAbs raised against chimeric forms of L985P protein molecule fused to Ra12 protein, either the long form (Ra12--which is the first 128 amino acids of Ra12) and/or the short form (Ra12S), or fused to a polyhistidine peptide or any combination of these molecules. Provided are the predicted cDNA and amino acid sequences for the his-tagged L985P-Ra12 fusion molecules: Ra12-L985P_cDNA (SEQ ID NO: 1875), Ra12-L985P_Protein (SEQ ID NO: 1876), Ra12S-L985P_cDNA (SEQ ID NO: 1877) and Ra12S-L985P_Protein (SEQ ID NO: 1878); and the L985P derived peptides: his-tagged Ra12S-L985PEx_cDNA (SEQ ID NO: 1879), his-tagged Ra12S-L985PEx_Protein (SEQ ID NO: 1880), L985P_Extracellular_Loop-2_cDNA (SEQ ID NO: 1881) and L985P_Extracellular_Loop-2_Peptide (SEQ ID NO: 1882).

PGPUB-DOCUMENT-NUMBER: 20020169304

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020169304 A1

TITLE: ASPARTYL PROTEASES

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|---------------|-------|---------|---------|
| KASER, MATTHEW R. | CASTRO VALLEY | CA | US | |
| Bandman, Olga | Mountain view | CA | US | |
| Hillman, Jennifer L. | Mountain view | CA | US | |
| Lal, Preeti | Santa clara | CA | US | |
| COCKS, BENJAMIN G. | SUNNYVALE | CA | US | |
| LORING, JEANNE | FORSTER CITY | CA | US | |
| TANG, Y. TOM | SAN JOSE | CA | US | |
| YUE, HENRY | SUNNYVALE | CA | US | |

APPL-NO: 09/ 470954

DATE FILED: December 22, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

US-CL-CURRENT: 536/23.2,435/252.3 ,435/320.1

ABSTRACT:

The invention provide mammalian nucleic acid molecules and fragments thereof. It also provides for the use of the mammalian nucleic acid molecules for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with Alzheimer's disease and Down syndrome. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid molecules.

----- KWIC -----

Detail Description Paragraph - DETX:

[0036] "Protein" refers to an amino acid sequence, oligopeptide, peptide, polypeptide or portions thereof whether naturally occurring or synthetic.

Detail Description Paragraph - DETX:

[0063] A multitude of nucleic acid molecules encoding ASP may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleic acid sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources that have been selected for their efficiency in a particular host. The vector, nucleic acid molecule, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

PGPUB-DOCUMENT-NUMBER: 20020168647

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168647 A1

TITLE: Compositions and methods for the therapy and diagnosis of head and neck cancer

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------|----------|-------|---------|---------|
| Wang, Tongtong | Medina | WA | US | |
| Fan, Liquin | Bellevue | WA | US | |

APPL-NO: 09/ 920455

DATE FILED: August 1, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60249933 20001116 US
non-provisional-of-provisional 60223281 20000803 US

US-CL-CURRENT: 435/6,435/183 ,435/7.23 ,530/388.26 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as head and neck cancer, are disclosed. Compositions may comprise one or more head and neck tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a head and neck tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as head and neck cancer. Diagnostic methods based on detecting a head and neck tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 60/249,933, filed Nov. 16, 2000 and 60/223,281, filed Aug. 3, 2000, which provisional applications are incorporated herein by reference in their entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0354] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0438] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020168637

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168637 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------|-------|---------|---------|
| Wang, Tongtong | Medina | WA | US | |
| Bangur, Chaitanya S. | Seattle | WA | US | |
| Lodes, Michael J. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Mannion, Jane | Edmonds | WA | US | |
| Fan, Liqun | Bellevue | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |

APPL-NO: 09/ 736457

DATE FILED: December 13, 2000

RELATED-US-APPL-DATA:

child 09736457 A1 20001213 parent continuation-in-part-of 09702705 20001030 US
PENDING child 09736457 A1 20001213 parent continuation-in-part-of 09677419
20001006 US PENDING child 09736457 A1 20001213 parent continuation-in-part-of
09671325 20000926 US PENDING child 09736457 A1 20001213 parent
continuation-in-part-of 09658824 20000908 US PENDING child 09736457 A1 20001213
parent continuation-in-part-of 09651563 20000829 US PENDING child 09736457 A1
20001213 parent continuation-in-part-of 09614124 20000711 US PENDING child
09736457 A1 20001213 parent continuation-in-part-of 09589184 20000605 US
PENDING child 09736457 A1 20001213 parent continuation-in-part-of 09560406
20000427 US PENDING child 09736457 A1 20001213 parent continuation-in-part-of
09546259 20000410 US PENDING child 09736457 A1 20001213 parent
continuation-in-part-of 09533077 20000322 US PENDING child 09736457 A1 20001213
parent continuation-in-part-of 09519642 20000306 US PENDING child 09736457 A1
20001213 parent continuation-in-part-of 09476300 19991230 US PENDING child
09736457 A1 20001213 parent continuation-in-part-of 09466867 19991217 US
PENDING child 09736457 A1 20001213 parent continuation-in-part-of 09419356
19991015 US PENDING child 09736457 A1 20001213 parent continuation-in-part-of
09346492 19990630 US PENDING child 09736457 A1 20001213 parent
continuation-in-part-of PCT/US00/18061 19990630 US UNKNOWN

US-CL-CURRENT: 435/6,435/183 ,435/320.1 ,435/325 ,435/69.1 ,435/7.23 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No. 09/702,705, filed Oct. 30, 2000; U.S. patent application Ser. No. 09/677,419, filed Oct. 6, 2000; U.S. patent application Ser. No. 09/671,325, filed Sep. 26, 2000; U.S. patent application Ser. No. 09/658,824, filed Sep. 8, 2000; U.S. patent application Ser. No. 09/651,563, filed Aug. 29, 2000; U.S. patent application Ser. No. 09/614,124, filed Jul. 11, 2000; U.S. patent application Ser. No. 09/589,184, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000; U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000; U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000; U.S. patent application Ser. No. 09/519,642, filed Mar. 6, 2000; U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999; U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999; U.S. patent application Ser. No. 09/419,356, filed Oct. 15, 1999; U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999; each a CIP of the previous application and all pending; and PCT/US00/18061, filed Jun. 30, 1999, pending.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0162] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020165371

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020165371 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|------------------|-------|---------|---------|
| Frudakis, Tony N. | Sarasota | FL | US | |
| Reed, Steven G. | Bellevue | WA | US | |
| Smith, John M. | Columbia Heights | MN | US | |
| Misher, Lynda E. | Seattle | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A.W. | Bellevue | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Day, Craig H. | Shoreline | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Deng, Ta | Edmonds | WA | US | |

APPL-NO: 09/ 924400

DATE FILED: August 7, 2001

RELATED-US-APPL-DATA:

child 09924400 A1 20010807 parent continuation-in-part-of 09810936 20010316 US
PENDING child 09810936 20010316 US parent continuation-in-part-of 09699295
20001026 US PENDING child 09699295 20001026 US parent continuation-in-part-of
09590583 20000608 US PENDING child 09590583 20000608 US parent
continuation-in-part-of 09577505 20000524 US PENDING child 09577505 20000524 US
parent continuation-in-part-of 09534825 20000323 US PENDING child 09534825
20000323 US parent continuation-in-part-of 09429755 19991028 US PENDING child
09429755 19991028 US parent continuation-in-part-of 09289198 19990409 US
PENDING child 09289198 19990409 US parent continuation-in-part-of 09062451
19980417 US GRANTED parent-patent 6344550 US child 09062451 19980417 US parent
continuation-in-part-of 08991789 19971211 US GRANTED parent-patent 6225054 US
child 08991789 19971211 US parent continuation-in-part-of 08838762 19970409 US
ABANDONED child 08838762 19970409 US parent a-371-of-international
PCT/US97/00485 19970110 WO UNKNOWN child 08700014 19960820 US parent
continuation-in-part-of 08585392 19960111 US ABANDONED

US-CL-CURRENT: 536/23.1,435/183 ,435/320.1 ,435/325 ,435/69.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/810,936, filed Mar. 16, 2001, which is a continuation in-part of U.S. patent application Ser. No. 09/699,295, filed Oct. 26, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/590,583, filed Jun. 8, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/577,505, filed May 24, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/534,825, filed Mar. 22, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/429,755, filed Oct. 28, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/289,198, filed Apr. 9, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/062,451, filed Apr. 17, 1998, which is a continuation in part of U.S. patent application Ser. No. 08/991,789, filed Dec. 11, 1997, which is a continuation-in-part of U.S. patent application Ser. No. 08/838,762, filed Apr. 9, 1997, now abandoned, which claims priority from International Patent Application No. PCT/US97/00485, filed Jan. 10, 1997, and is a continuation-in-part of U.S. patent application Ser. No. 08/700,014, filed Aug. 20, 1996, which is a continuation-in-part of U.S. patent application Ser. No. 08/585,392, filed Jan. 11, 1996, now abandoned.

----- KWIC -----

Detail Description Paragraph - DETX:

[0174] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020164345

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164345 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------|------|-------|---------|---------|
| Jiang, Yuqiu | Kent | WA | US | |

APPL-NO: 10/ 042125

DATE FILED: October 18, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60242321 20001020 US

US-CL-CURRENT: 424/185.1,435/183 ,435/320.1 ,435/325 ,435/69.3 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial No. 60/242,321, filed Oct. 20, 2000, incorporated herein in its entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0208] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression

of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020161215

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020161215 A1

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|-----------|-------|---------|---------|
| Daggett, Lorrie P. | San Diego | CA | US | |
| Ellis, Steven B. | San Diego | CA | US | |
| Liaw, Chen Wang | San Diego | CA | US | |
| Lu, Chin-Chun | San Diego | CA | US | |

APPL-NO: 09/ 945901

DATE FILED: September 4, 2001

RELATED-US-APPL-DATA:

child 09945901 A1 20010904 parent division-of 08940035 19970929 US GRANTED
parent-patent 6316611 US child 08940035 19970929 US parent division-of 08231193
19940420 US GRANTED parent-patent 5849895 US child 08231193 19940420 US parent
continuation-in-part-of 08052449 19930420 US ABANDONED

US-CL-CURRENT: 536/23.5,435/320.1 ,435/325 ,435/69.1 ,530/350

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and

modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

[0001] This application is a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now pending.

----- KWIC -----

Detail Description Paragraph - DETX:

[0062] As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detail Description Paragraph - DETX:

[0083] Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA

sequences which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

PGPUB-DOCUMENT-NUMBER: 20020161193

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020161193 A1

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|-----------|-------|---------|---------|
| Daggett, Lorrie P. | San Diego | CA | US | |
| Lu, Chin-Chun | San Diego | CA | US | |

APPL-NO: 10/ 007747

DATE FILED: December 7, 2001

RELATED-US-APPL-DATA:

child 10007747 A1 20011207 parent division-of 09648797 20000828 US PENDING
child 09648797 20000828 US parent division-of 08231193 19940420 US GRANTED
parent-patent 5849895 US child 08231193 19940420 US parent
continuation-in-part-of 08052449 19930420 US ABANDONED

US-CL-CURRENT: 530/350,435/320.1 ,435/325 ,435/69.1 ,536/23.5

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA

receptor subunits.

[0001] This application is a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now pending.

----- KWIC -----

Detail Description Paragraph - DETX:

[0059] As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3' 1'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detail Description Paragraph - DETX:

[0080] Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

PGPUB-DOCUMENT-NUMBER: 20020156011

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020156011 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|----------|-------|---------|---------|
| Jiang, Yuqiu | Kent | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Secrist, Heather | Seattle | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Stolk, John A. | Bothell | WA | US | |

APPL-NO: 10/ 046935

DATE FILED: January 15, 2002

RELATED-US-APPL-DATA:

child 10046935 A1 20020115 parent continuation-in-part-of 09878178 20010608 US
PENDING non-provisional-of-provisional 60270216 20010220 US
non-provisional-of-provisional 60210899 20000609 US

US-CL-CURRENT: 514/12,514/44 ,530/350 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0159] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter

polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020155571

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020155571 A1

TITLE: Triazine degrading enzymes

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|---------------|-------|---------|---------|
| Raillard, Sun Ai | Mountain View | CA | US | |
| Minshull, Jeremy | Minshull | CA | US | |
| Gustafsson, Claes | Belmont | CA | US | |

APPL-NO: 09/ 796990

DATE FILED: February 28, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60185809 20000229 US

US-CL-CURRENT: 435/196,435/121 ,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

New triazine hydrolases, (both nucleic acids and proteins) are provided. Compositions which include these new proteins and/or genes, recombinant cells, shuffling methods involving the new triazine hydrolases, antibodies to the new triazine hydrolases and methods of using the triazine hydrolases are also provided.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Pursuant to 35 U.S.C. .sctn. 119(e) and any other applicable statute or rule, the present application claims benefit of and priority to U.S. Ser. No. 60/185,809 "Triazine Degrading Enzymes," by Bermudez et al., filed Feb. 29, 2000, and co-filed PCT application, "Triazine Degrading Enzymes," by Bermudez et al., filed Feb. 27, 2001, Attorney Docket No. 02-104510PC.

----- KWIC -----

Detail Description Paragraph - DETX:

[0061] The polynucleotide sequences of the present invention are optionally

engineered in order to alter a triazine hydrolase coding sequence, for a variety of reasons, including, but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis or de novo synthesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Detail Description Paragraph - DETX:

[0067] The polynucleotides of the present invention may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adenovirus, adeno-associated virus, retroviruses, agrobacterium, and many others. Any vector that transduces genetic material into a cell, and, if replication is desired, which is replicable and viable in the relevant host can be used depending on where expression is desired.

Detail Description Paragraph - DETX:

[0178] In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example, recombinant protein may be produced in a mammalian cell line. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see, Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity). Alternatively, one or more synthetic or recombinant polypeptide derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

PGPUB-DOCUMENT-NUMBER: 20020150922

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020150922 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|----------|-------|---------|---------|
| Stolk, John A. | Bothell | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Chenault, Ruth A. | Seattle | WA | US | |
| Meagher, Madeleine Joy | Seattle | WA | US | |

APPL-NO: 09/ 998598

DATE FILED: November 16, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60304037 20010710 US
non-provisional-of-provisional 60279670 20010328 US
non-provisional-of-provisional 60267011 20010206 US
non-provisional-of-provisional 60252222 20001120 US

US-CL-CURRENT: 435/6,435/183 ,435/320.1 ,435/325 ,435/69.1 ,435/7.23 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[2761] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression

of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020150581

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020150581 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------|-------|---------|---------|
| Jiang, Yuqiu | Kent | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Durham, Margarita | Seattle | WA | US | |

APPL-NO: 10/ 007805

DATE FILED: December 7, 2001

RELATED-US-APPL-DATA:

child 10007805 A1 20011207 parent continuation-in-part-of 09834759 20010413 US
PENDING child 09834759 20010413 US parent continuation-in-part-of 09620405
20000720 US PENDING child 09620405 20000720 US parent continuation-in-part-of
09604287 20000622 US PENDING child 09604287 20000622 US parent
continuation-in-part-of 09590751 20000608 US PENDING child 09590751 20000608 US
parent continuation-in-part-of 09551621 20000417 US PENDING child 09551621
20000417 US parent continuation-in-part-of 09433826 19991103 US PENDING child
09433826 19991103 US parent continuation-in-part-of 09389681 19990902 US
PENDING child 09389681 19990902 US parent continuation-in-part-of 09339338
19990623 US PENDING child 09339338 19990623 US parent continuation-in-part-of
09285480 19990402 US PENDING child 09285480 19990402 US parent
continuation-in-part-of 09222575 19981228 US GRANTED parent-patent 6387697 US

US-CL-CURRENT: 424/155.1,435/183 ,435/320.1 ,435/325 ,435/6 ,435/69.1
,435/7.23 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more

breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation-In-Part of U.S. patent application Ser. No. 09/834,759, filed Apr. 13, 2001 which is a Continuation-In-Part of U.S. patent application Ser. No. 09/620,405, filed Jul. 20, 2000, which is a Continuation-In-Part of U.S. patent application Ser. No. 09/604,287, filed Jun. 22, 2000, which is a Continuation-In-Part of U.S. patent application Ser. No. 09/590,751, filed Jun. 8, 2000, which is a Continuation-In-Part Of U.S. patent application Ser. No. 09/551,621, filed Apr. 17, 2000, which is a Continuation-In-Part of U.S. patent application Ser. No. 09/433,826, filed on Nov. 3, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/389,681, filed on Sep. 2, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/339,338, filed on Jun. 23, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/285,480, filed on Apr. 2, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/222,575, filed Dec. 28, 1998.

----- KWIC -----

Detail Description Paragraph - DETX:

[0698] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020147143

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020147143 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|---------------|-------|---------|---------|
| Wang, Tongtong | Medina | WA | US | |
| Durham, Margarita | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Watanabe, Yoshihiro | Mercer Island | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Peckham, David W. | Seattle | WA | US | |
| Fanger, Neil | Seattle | WA | US | |

APPL-NO: 09/ 897778

DATE FILED: June 28, 2001

RELATED-US-APPL-DATA:

child 09897778 A1 20010628 parent continuation-in-part-of 09850716 20010507 US
PENDING child 09850716 20010507 US parent continuation-in-part-of 09735705
20001212 US PENDING child 09735705 20001212 US parent continuation-in-part-of
09685696 20001009 US PENDING child 09685696 20001009 US parent
continuation-in-part-of 09662786 20000915 US PENDING child 09662786 20000915 US
parent continuation-in-part-of 09643597 20000821 US PENDING child 09643597
20000821 US parent continuation-in-part-of 09630940 20000802 US PENDING child
09630940 20000802 US parent continuation-in-part-of 09606421 20000628 US
PENDING child 09606421 20000628 US parent continuation-in-part-of 09542615
20000404 US PENDING child 09542615 20000404 US parent continuation-in-part-of
09510376 20000222 US PENDING child 09510376 20000222 US parent
continuation-in-part-of 09480884 20000110 US PENDING child 09480884 20000110 US
parent continuation-in-part-of 09476496 19991230 US PENDING child 09476496
19991230 US parent continuation-in-part-of 09466396 19991217 US PENDING child
09466396 19991217 US parent continuation-in-part-of 09285479 19990402 US
PENDING child 09285479 19990402 US parent continuation-in-part-of 09221107
19981222 US PENDING child 09221107 19981222 US parent continuation-in-part-of
09123912 19980727 US PATENTED child 09123912 19980727 US parent
continuation-in-part-of 09040802 19980318 US PENDING

US-CL-CURRENT: 514/12,435/183 ,435/320.1 ,435/325 ,435/69.1 ,514/44 ,530/350
,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/850,716 filed May 7, 2001; which is a continuation-in-part of U.S. patent application Ser. No. 09/735,705 filed Dec. 12, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/685,696 filed Oct. 9, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/662,786 filed Sep. 15, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/643,597 filed Aug. 21, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/630,940 filed Aug. 2, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/606,421 filed Jun. 28, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/542,615 filed Apr. 4, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/510,376 filed Feb. 22, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/480,884 filed Jan. 10, 2000; which is a continuation-in-part of U.S. patent application Ser. No. 09/476,496 filed Dec. 30, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/466,396 filed Dec. 17, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/285,479 filed Apr. 2, 1999; which is a continuation-in-part of U.S. patent application Ser. No. 09/221,107 filed Dec. 22, 1998; which is a continuation-in-part of U.S. patent application Ser. No. 09/123,912 filed Jul. 27, 1998; which is a continuation-in-part of U.S. patent application Ser. No. 09/040,802 filed Mar. 18, 1998 and all incorporated by reference herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0543] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020146776

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020146776 A1

TITLE: Compounds and methods for treatment and diagnosis of chlamydial infection

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------|---------|-------|---------|---------|
| Bhatia, Ajay | Seattle | WA | US | |
| Probst, Peter | Seattle | WA | US | |

APPL-NO: 10/ 007693

DATE FILED: December 5, 2001

RELATED-US-APPL-DATA:

child 10007693 A1 20011205 parent continuation-in-part-of 09841260 20010423 US
PENDING non-provisional-of-provisional 60219752 20000720 US
non-provisional-of-provisional 60198853 20000421 US

US-CL-CURRENT: 435/69.3,435/183 ,435/252.3 ,435/320.1 ,536/23.7

ABSTRACT:

Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a Chlamydia antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0212] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and

PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site-directed** mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0296] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, **synthetic antisense nucleotide sequences** will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020146727

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020146727 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|-------------------|-------|---------|---------|
| Dillon, Davin C. | Issaquah | WA | US | |
| Day, Craig H. | Shoreline | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Bennigton, Angela Ann | Seattle | WA | US | |
| Zehentner, Barbara | Bainbridge Island | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |

APPL-NO: 10/ 010742

DATE FILED: November 30, 2001

RELATED-US-APPL-DATA:

child 10010742 A1 20011130 parent continuation-in-part-of 09910689 20010720 US
PENDING child 09910689 20010720 US parent continuation-in-part-of 09778320
20010206 US PENDING child 09778320 20010206 US parent continuation-in-part-of
09571025 20000515 US PENDING child 09571025 20000515 US parent
continuation-in-part-of 09545068 20000407 US ABANDONED child 09545068 20000407
US parent continuation-in-part-of 09523586 20000310 US ABANDONED child 09523586
20000310 US parent continuation-in-part-of 09510662 20000222 US PENDING child
09510662 20000222 US parent continuation-in-part-of 09451651 19991130 US
PENDING

US-CL-CURRENT: 435/6,435/183 ,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such

polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/910,689, filed Jul. 20, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/778,320, filed Feb. 6, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/571,025, filed May 15, 2000 (abandoned), which is a continuation-in-part of U.S. patent application Ser. No. 09/545,068, filed Apr. 7, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/523,586, filed Mar. 10, 2000 (abandoned), which is a continuation-in-part of U.S. patent application Ser. No. 09/510,662, filed Feb. 22, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/451,651, filed Nov. 30, 1999, each of which is incorporated in their entirety herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0372] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020142957

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020142957 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|----------|-------|---------|---------|
| Hepler, William T. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Pyle, Ruth A. | Seattle | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |

APPL-NO: 09/ 924401

DATE FILED: August 7, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60223826 20000808 US

non-provisional-of-provisional 60223653 20000807 US

US-CL-CURRENT: 514/12,435/320.1 ,435/325 ,435/69.3 ,435/7.23 ,514/44 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Patent Application No. 60/223,826 filed Aug. 8, 2000 and 60/223,653 filed Aug., 7, 2000 where both these provisional applications are incorporated herein by reference in their entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0161] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0241] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020137911

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137911 A1

TITLE: Compositions and methods for the therapy and diagnosis of pancreatic cancer

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------|----------|-------|---------|---------|
| Pyle, Ruth A. | Seattle | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |

APPL-NO: 09/ 919344

DATE FILED: July 30, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60234451 20000921 US
non-provisional-of-provisional 60223102 20000803 US

US-CL-CURRENT: 536/23.2,435/183 ,435/320.1 ,435/325 ,435/69.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as pancreatic cancer, are disclosed. Compositions may comprise one or more pancreatic tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a pancreatic tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as pancreatic cancer. Diagnostic methods based on detecting a pancreatic tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 60/234,451 filed Sep. 21, 2000 and 60/223,102 filed Aug. 3, 2000, where these two provisional applications are incorporated herein by reference in their entireties.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0104] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0183] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, **synthetic antisense nucleotide sequences** will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020137889

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137889 A1

TITLE: Stress resistant retroviruses

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|--------------|-------|---------|---------|
| Soong, Nay Wei | San Jose | CA | US | |
| Stemmer, Willem P.C. | Los Gatos | CA | US | |
| Powell, Sharon K. | Alameda | CA | US | |
| Otto, Edward | Falls Church | VA | US | |

APPL-NO: 09/ 954983

DATE FILED: September 17, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60233398 20000918 US

US-CL-CURRENT: 530/350,435/235.1 ,435/325 ,435/69.1 ,536/23.72

ABSTRACT:

Stress and/or shear resistant retrovirus envelope protein polypeptides and nucleic acids encoding such polypeptides, as well as fragments of such nucleic acids and polypeptides and compositions thereof, are provided. Retroviruses incorporating such polypeptides and methods of using stress resistant retrovirus envelope protein polypeptides and corresponding nucleic acids are also described.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Patent Application Ser. No. 60/233,398 filed Sep. 18, 2000, the disclosure of which is incorporated herein by reference in its entirety for all purposes.

----- KWIC -----

Detail Description Paragraph - DETX:

[0147] The polynucleotide sequences of the present invention can be engineered in order to alter an envelope protein coding sequence for a variety of reasons,

including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques that are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Detail Description Paragraph - DETX:

[0153] The polynucleotides of the present invention may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adenovirus, adeno-associated virus, retroviruses and many others. Any vector that transduces genetic material into a cell, and, if replication is desired, that is replicable and viable in the relevant host can be used.

PGPUB-DOCUMENT-NUMBER: 20020137214

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137214 A1

TITLE: Marker free transgenic plants engineering the chloroplast genome without the use of antibiotic selection

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------|-------------|-------|---------|---------|
| Daniell, Henry | Winter Park | FL | US | |

APPL-NO: 09/ 807722

DATE FILED: April 18, 2001

PCT-DATA:

APPL-NO: PCT/US01/06275

DATE-FILED: Feb 28, 2001

PUB-NO:

PUB-DATE:

371-DATE:

102(E)-DATE:

US-CL-CURRENT: 435/468

ABSTRACT:

The present invention provides for a method to circumvent the problem of using antibiotic resistant selectable markers. In particular, target plants are transformed using a plastid vector which contains heterologous DNA sequences coding for a phytotoxin detoxifying enzyme or protein. The selection process involves converting an antibiotic-free phytotoxic agent by the expressed phytotoxin detoxifying enzyme or protein to yield a nontoxic compound. The invention provides for various methods to use antibiotic-free selection in chloroplast transformation.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Application Nos. 60/208,763, filed Jun. 06, 2000, No. 60/257,406, filed Dec. 22, 2000 and No. 60/259,154, filed Dec. 28, 2000, Ser. No. 60/186,308, filed Mar. 02, 2000. All applications are hereby incorporated by reference.

----- KWIC -----

Detail Description Paragraph - DETX:

[0067] The BADH gene expression was tested in E. coli cell extracts by enzyme assays before proceeding with bombardment. The universal vector pLD-BADH was transformed into the E. coli strain XL-1 Blue and grown in Terrific Broth (Guda et al. 2000) in the presence of ampicillin (100 .mu.g/ml) at 37.degree. C. for 24 hours. In E. coli, the level of expression by the chloroplast Prrn promoter is equivalent to that of the highly efficient T7 promoter and both systems have highly compatible protein synthetic machinery (Brixey et al. 1997). Therefore, BADH enzyme activity was tested in untransformed cells and cells transformed with pLD-BADH, a high copy number plasmid (FIG. 2). Crude sonic extracts isolated from transformed cells showed 3-5 fold more BADH activity than the untransformed control, confirming that the expression cassette is fully functional. This result also suggests that codon preference of the nuclear BADH gene is compatible with expression in the prokaryotic chloroplast compartment.

Detail Description Paragraph - DETX:

[0099] Targeted Genes of Interest include: Polypeptide pro-insulin, PBP synthetic polymer, Insulin, Human Serum Albumin, and Herbicide glyphosate. Other genes of interest include, but are not limited to the aminoglycosides listed in "Aminoglycosides: A Practical Review" by Gonzalez, L. S. and Spencer, J. P., American Family Physician, No. 8, 58:1811.

PGPUB-DOCUMENT-NUMBER: 20020136728

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020136728 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|----------|-------|---------|---------|
| King, Gordon E. | Seattle | WA | US | |
| Meagher, Madeleine Joy | Seattle | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Secrist, Heather | Seattle | WA | US | |

APPL-NO: 09/ 920300

DATE FILED: July 31, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60302051 20010629 US
non-provisional-of-provisional 60279763 20010328 US
non-provisional-of-provisional 60223283 20000803 US

US-CL-CURRENT: 424/155.1,435/183 ,435/320.1 ,435/6 ,435/69.1 ,435/7.23
,530/350 ,530/388.8 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/302,051 filed Jun. 29, 2001, U.S. Provisional Application No. 60/279,763 filed Mar. 28, 2001, and U.S. Provisional Application No. 60/223,283 filed Aug. 3, 2000, all incorporated in their entirety herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0152] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020131971

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020131971 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|-----------|-------|---------|---------|
| King, Gordon E. | Shoreline | WA | US | |
| Meagher, Madeleine Joy | Seattle | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Secrist, Heather | Seattle | WA | US | |

APPL-NO: 10/ 033528

DATE FILED: December 26, 2001

RELATED-US-APPL-DATA:

child 10033528 A1 20011226 parent continuation-in-part-of 09920300 20010731 US
PENDING non-provisional-of-provisional 60302051 20010629 US
non-provisional-of-provisional 60279763 20010328 US
non-provisional-of-provisional 60223283 20000803 US

US-CL-CURRENT: 424/155.1,435/183 ,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[2046] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression

of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020123619

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020123619 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: September 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|---------|-------|---------|---------|
| Benson, Darin R. | Seattle | WA | US | |
| Mohamath, Raodoh | Seattle | WA | US | |
| Lodes, Michael J. | Seattle | WA | US | |

APPL-NO: 09/ 960253

DATE FILED: September 20, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60234837 20000922 US
non-provisional-of-provisional 60239440 20001010 US
non-provisional-of-provisional 60301928 20010629 US

US-CL-CURRENT: 536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as lung cancer, are disclosed. Compositions may comprise one or more lung tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a lung tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as lung cancer. Diagnostic methods based on detecting a lung tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Patent Applications No. 60/234,837 filed Sep. 22, 2000, No. 60/239,440 filed Oct. 10, 2001, and No. 60/301,928 filed Jun. 29, 2001, and are herewith incorporated in their entirety by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0193] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020119531

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020119531 A1

TITLE: Prostate-associated protease antibody

PUBLICATION-DATE: August 29, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------|---------------|-------|---------|---------|
| Bandman, Olga | Mountain View | CA | US | |
| Lal, Preeti G. | Santa Clara | CA | US | |

APPL-NO: 09/ 988975

DATE FILED: November 19, 2001

RELATED-US-APPL-DATA:

child 09988975 A1 20011119 parent continuation-in-part-of 09478957 20000107 US
GRANTED parent-patent 6350448 US child 09988975 A1 20011119 parent
continuation-in-part-of 08807151 19970227 US GRANTED parent-patent 6043033 US

US-CL-CURRENT: 435/70.21,435/183 ,530/388.26

ABSTRACT:

The present invention provides a human prostate-associated protease (HUPAP), polynucleotides which encode HUPAP and antibodies which specifically bind HUPAP. The invention also provides expression vectors, host cells, agonists, antagonists, and antisense molecules. The invention also provides methods for producing and using HUPAP and for treating disorders associated with expression of HUPAP.

[0001] This application is a continuation-in-part of U.S. Ser. No. 09/478,957, filed Jan. 7, 2000, and U.S. Ser. No. 08/807,151, filed Feb. 27, 1997, which issued as U.S. Pat. No. 6,043,033, on Mar. 28, 2000.

----- KWIC -----

Detail Description Paragraph - DETX:

[0045] "Protein" refers to an amino acid sequence, peptide, or polypeptide, and portions thereof, that are naturally occurring, recombinantly produced, or synthetic. "HUPAP" refers to a purified protein obtained from any species, particularly mammalian including bovine, equine, murine, ovine, porcine, and

preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

Detail Description Paragraph - DETX:

[0061] Any one of a multitude of polynucleotides encoding the HUPAP may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The polynucleotide can be engineered by such methods as DNA shuffling, as described in U.S. Pat. No. 5,830,721, and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change **codon preference** to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (**promoters**, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources which have been selected for their efficiency in a particular host. The vector, polynucleotide, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

PGPUB-DOCUMENT-NUMBER: 20020115139

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020115139 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|------------|-------|---------|---------|
| Kalos, Michael D. | Seattle | WA | US | |
| McNeill, Patricia D. | Des Moines | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |

APPL-NO: 09/ 850716

DATE FILED: May 7, 2001

RELATED-US-APPL-DATA:

child 09850716 A1 20010507 parent continuation-in-part-of 09735705 20001212 US
PENDING

US-CL-CURRENT: 435/69.1,435/183 ,435/320.1 ,435/325 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. patent application Ser. Nos. 09/735,705, filed Dec. 12, 2000; 09/685,696, filed Oct. 9, 2000; 09/662,786, filed Sep. 15, 2000; 09/643,597, filed Aug. 21, 2000; 09/630,940 filed Aug. 2, 2000; 09/606,421 filed Jun. 28, 2000; 09/542,615 filed Apr. 4, 2000; 09/510,376 filed Feb. 22, 2000; 09/480,884 filed Jan. 10, 2000; 09/476,496 filed Dec. 30, 1999; 09/466,396 filed Dec. 17, 1999; 09/285,479 filed Apr. 2, 1999; 09/221,107 filed Dec. 22, 1998; 09/123,912 filed Jul. 27, 1998; 09/040,802 filed Mar. 18, 1998; each a CIP of the previous application and pending, and all incorporated by reference herein.

----- KWIC -----

Detail Description Paragraph - DETX:

[0517] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020111308

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020111308 A1

TITLE: Compositions and methods for the therapy and diagnosis of pancreatic cancer

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------|----------|-------|---------|---------|
| Pyle, Ruth A. | Seattle | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |

APPL-NO: 09/ 920345

DATE FILED: July 31, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60222886 20000803 US

US-CL-CURRENT: 514/12,435/183 ,435/320.1 ,435/325 ,435/69.1 ,514/44 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as pancreatic cancer, are disclosed. Compositions may comprise one or more pancreatic tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a pancreatic tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as pancreatic cancer. Diagnostic methods based on detecting a pancreatic tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/222,886 filed Aug. 3, 2000, incorporated herein by reference in its entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0101] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0180] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, **synthetic antisense nucleotide sequences** will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020110832

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020110832 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------|----------|-------|---------|---------|
| Pyle, Ruth A. | Seattle | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Secrist, Heather | Seattle | WA | US | |

APPL-NO: 09/ 919580

DATE FILED: July 30, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60302702 20010703 US
non-provisional-of-provisional 60277495 20010320 US
non-provisional-of-provisional 60237406 20001002 US
non-provisional-of-provisional 60223265 20000803 US

US-CL-CURRENT: 435/7.1,530/350 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Nos. 60/302,702 filed Jul. 3, 2001, 60/277,495 filed Mar. 20, 2001, 60/237,406 filed Oct. 2, 2000, and 60/223,265 filed Aug. 3, 2000, all incorporated in their entirety herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0151] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020110563

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020110563 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|-------------------|-------|---------|---------|
| Reed, Steven G. | Bellevue | WA | US | |
| Lodes, Michael J. | Seattle | WA | US | |
| Mohamath, Raodoh | Seattle | WA | US | |
| Secrist, Heather | Seattle | WA | US | |
| Benson, Darin R. | Seattle | WA | US | |
| Indirias, Carol Yoseph | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Fling, Steven P. | Bainbridge Island | WA | US | |
| Algate, Paul A. | Issaquah | WA | US | |
| Elliott, Mark | Seattle | WA | US | |
| Mannion, Jane | Edmonds | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |

APPL-NO: 09/ 738973

DATE FILED: December 14, 2000

RELATED-US-APPL-DATA:

child 09738973 A1 20001214 parent continuation-in-part-of 09704512 20001101 US
PENDING

US-CL-CURRENT: 424/155.1,435/183 ,435/320.1 ,435/325 ,435/6 ,435/69.1
,435/7.23 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No.

09/704,512, filed Nov. 1, 2000; U.S. patent application Ser. No. _____, filed Sep. 20, 2000; U.S. patent application Ser. No. 09/640,878, filed Aug. 18, 2000; U.S. patent application Ser. No. 09/588,937, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/538,037, filed Mar. 29, 2000; U.S. patent application Ser. No. 09/518,809, filed Mar. 3, 2000; U.S. patent application Ser. No. 09/476,235 filed Dec. 30, 1999; U.S. patent application Ser. No. 09/370,838, filed Aug. 9, 1999; and U.S. patent application Ser. No. 09/285,323, filed Apr. 2, 1999, each a CIP of the previous application and all pending, and PCT/US00/08560, filed Mar. 30, 2000, pending.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0734] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020102679

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020102679 A1

TITLE: Compositions and methods for the therapy and diagnosis of ovarian cancer

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Secrist, Heather | Seattle | WA | US | |
| Lodes, Michael J. | Seattle | WA | US | |
| Algate, Paul A. | Issaquah | WA | US | |
| Fling, Steven P. | Bainbridge Island | WA | US | |
| Mannion, Jane | Edmonds | WA | US | |
| Benson, Darin R. | Seattle | WA | US | |
| Carter, Darrick | Seattle | WA | US | |

APPL-NO: 09/ 864864

DATE FILED: May 23, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60211457 20000613 US
non-provisional-of-provisional 60207107 20000524 US
non-provisional-of-provisional 60213673 20000621 US
non-provisional-of-provisional 60223288 20000803 US
non-provisional-of-provisional 60272790 20010301 US

US-CL-CURRENT: 435/183,435/320.1 ,435/325 ,435/69.1 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses an ovarian tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Diagnostic methods based on detecting an ovarian tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Applications 60/211,457 filed Jun. 13, 2000; 60/207,107 filed May 24, 2000; 60/213,673 filed Jun. 21, 2000; 60/223,288 filed Aug. 3, 2000; and 60/272,790 filed Mar. 1, 2001, incorporated in their entirety herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX:

[0066] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX:

[0149] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020102615

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020102615 A1

TITLE: Transcription factor regulatory protein

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|---------------|-------|---------|---------|
| Kaser, Matthew R. | Castro Valley | CA | US | |
| Baughn, Mariah R. | San Leandro | CA | US | |

APPL-NO: 09/ 836941

DATE FILED: April 17, 2001

RELATED-US-APPL-DATA:

child 09836941 A1 20010417 parent division-of 09286132 19990401 US GRANTED
parent-patent 6242185 US

US-CL-CURRENT: 435/7.9,435/183 ,435/6 ,435/70.21 ,530/388.1

ABSTRACT:

The invention provides a mammalian nucleic acid sequence and fragments thereof. It also provides for the use of these nucleic acid sequences in a model system for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with expression of the mammalian nucleic acid sequence. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid sequence.

[0001] This application is a divisional application of U.S. application Ser. No. 09/286,132 filed Apr. 1, 1999, all of which application is hereby incorporated by reference herein.

----- KWIC -----

Detail Description Paragraph - DETX:

[0029] "Polypeptide" refers to an amino acid, amino acid sequence, oligopeptide, peptide, or protein or portions thereof whether naturally occurring or synthetic.

Detail Description Paragraph - DETX:

[0051] A multitude of polynucleotide sequences capable of encoding the mammalian protein may be cloned into a vector and used to express the protein, or portions thereof, in appropriate host cells. The nucleotide sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain appropriate transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and 3' untranslated regions) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid sequence, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

PGPUB-DOCUMENT-NUMBER: 20020099012

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020099012 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: July 25, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|---------------|-------|---------|---------|
| Wang, Tongtong | Medina | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Watanabe, Yoshihiro | Mercer Island | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |

APPL-NO: 09/ 895828

DATE FILED: June 28, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60215696 20000629 US
non-provisional-of-provisional 60227142 20000822 US
non-provisional-of-provisional 60230481 20000906 US
non-provisional-of-provisional 60257729 20001221 US

US-CL-CURRENT: 514/12,435/183 ,435/320.1 ,435/325 ,435/6 ,435/69.1 ,530/350 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as lung cancer, are disclosed. Compositions may comprise one or more lung tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a lung tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as lung cancer. Diagnostic methods based on detecting a lung tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Patent Application No. 60/215,696 filed Jun. 29, 2000; U.S. Patent Application No. 60/227,142 filed Aug. 22, 2000; U.S. Patent Application No. 60/230,481 filed Sep. 6, 2000 and

U.S. Patent Application No. 60/257,729 filed Dec. 21, 2000, which are incorporated by reference herein in their entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0541] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0625] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020090610

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020090610 A1

TITLE: Compositions and methods for the diagnosis and treatment of herpes simplex virus infection

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------|----------|-------|---------|---------|
| Hosken, Nancy A. | Seattle | WA | US | |
| Day, Craig H. | Seattle | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| McGowan, Patrick | Seattle | WA | US | |
| Sleath, Paul R. | Seattle | WA | US | |

APPL-NO: 09/ 894998

DATE FILED: June 28, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60277438 20010320 US

non-provisional-of-provisional 60215458 20000629 US

US-CL-CURRENT: 435/5,424/231.1 ,435/6 ,530/350 ,536/23.72

ABSTRACT:

Compounds and methods for the diagnosis and treatment of HSV infection are provided. The compounds comprise polypeptides that contain at least one antigenic portion of an HSV polypeptide and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits are also provided comprising such polypeptides and/or DNA sequences and a suitable detection reagent for the detection of HSV infection in patients and in biological samples.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/277,438 filed Mar. 20, 2001 and U.S. Provisional Application No. 60/215,458 filed Jun. 29, 2000 and are incorporated in their entirety by reference herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0121] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0205] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, **synthetic antisense nucleotide sequences** will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020086303

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020086303 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|-----------|-------|---------|---------|
| Meagher, Madeleine Joy | Seattle | WA | US | |
| King, Gordon E. | Shoreline | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Secrist, Heather | Seattle | WA | US | |

APPL-NO: 09/ 878134

DATE FILED: June 7, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60210667 20000609 US

non-provisional-of-provisional 60252614 20001122 US

US-CL-CURRENT: 435/6,424/155.1 ,435/183 ,435/320.1 ,435/325 ,435/69.1
,435/7.23 ,514/44 ,530/388.8 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0524] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not

limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site-**directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference,** produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020085998

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020085998 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|----------|-------|---------|---------|
| Jiang, Yuqiu | Kent | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |

APPL-NO: 09/ 834759

DATE FILED: April 13, 2001

RELATED-US-APPL-DATA:

child 09834759 A1 20010413 parent continuation-in-part-of 09620405 20000720 US
PENDING child 09620405 20000720 US parent continuation-in-part-of 09604287
20000622 US PENDING child 09604287 20000622 US parent continuation-in-part-of
09590751 20000608 US PENDING child 09590751 20000608 US parent
continuation-in-part-of 09551621 20000417 US PENDING child 09551621 20000417 US
parent continuation-in-part-of 09433826 19991103 US PENDING child 09433826
19991103 US parent continuation-in-part-of 09389681 19990902 US PENDING child
09389681 19990902 US parent continuation-in-part-of 09339338 19990623 US
PENDING child 09339338 19990623 US parent continuation-in-part-of 09285480
19990402 US PENDING child 09285480 19990402 US parent continuation-in-part-of
09222575 19981228 US PENDING

US-CL-CURRENT: 424/93.21,435/183 ,435/320.1 ,435/325 ,435/69.1 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This Application is a Continuation-In-Part of U.S. patent application Ser. No. 09/620,405, filed Jul. 20, 2000, which is A Continuation-In-Part of U.S. patent application Ser. No. 09/604,287, filed Jun. 22, 2000, which is a Continuation-In-Part of U.S. patent application Ser. No. 09/590,751, filed Jun. 8, 2000, which is a Continuation-In-Part Of U.S. patent application Ser. No. 09/551,621, filed Apr. 17, 2000, which is a Continuation-In-Part of U.S. patent application Ser. No. 09/433,836, filed on Nov. 3, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/389,681, filed on Sep. 2, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/339,338, filed on Jun. 23, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/285,480, filed on Apr. 2, 1999, which is a Continuation-In-Part of U.S. application Ser. No. 09/222,575, filed Dec. 28, 1998.

----- KWIC -----

Detail Description Paragraph - DETX:

[0661] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020082207

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020082207 A1

TITLE: Compositions and methods for the therapy and diagnosis of pancreatic cancer

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|----------|-------|---------|---------|
| Hirst, Shannon K. | Kirkland | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |

APPL-NO: 09/ 872153

DATE FILED: May 31, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60291197 20010515 US
non-provisional-of-provisional 60248980 20001114 US
non-provisional-of-provisional 60210329 20000607 US

US-CL-CURRENT: 514/12,435/183 ,435/320.1 ,435/325 ,435/6 ,435/69.1 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly pancreatic cancer, are disclosed. Illustrative compositions comprise one or more pancreatic tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly pancreatic cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/291,197 filed May 15, 2001, U.S. Provisional Application No. 60/248,980 filed Nov. 14, 2000, and U.S. Provisional Application No. 60/210,329 filed Jun. 7, 2000, incorporated in their entirety herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0173] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020081680

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020081680 A1

TITLE: Compositions and methods for the therapy and diagnosis of prostate cancer

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Stolk, John A. | Bothell | WA | US | |
| Day, Craig H. | Seattle | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A. W. | Bellevue | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Hural, John | Bainbridge Island | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |
| de Bassols, Carlota | Rixensart | | BE | |

APPL-NO: 09/ 822827

DATE FILED: March 28, 2001

RELATED-US-APPL-DATA:

child 09822827 A1 20010328 parent continuation-in-part-of 09780669 20010209 US
PENDING child 09780669 20010209 US parent continuation-in-part-of 09679272
20001004 US PENDING non-provisional-of-provisional 60157455 20000417 US

US-CL-CURRENT: 435/183,435/320.1 ,435/325 ,435/69.7 ,530/350 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly

prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis prevention and/or treatment of diseases, particularly prostate cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/679,272, filed Oct. 4, 2000, which claims priority to U.S. Provisional Application No. 60/157,455, filed Oct. 4, 1999; and is related to U.S. patent application Ser. No. 09/759,143, filed Jan. 12, 2001, U.S. patent application Ser. No. 09/709,729, filed Nov. 9, 2000, U.S. patent application Ser. No. 09/685,166, filed Oct. 10, 2000, U.S. patent application Ser. No. 09/679,426, filed Oct. 2, 2000, U.S. patent application Ser. No. 09/657,279, filed Sep. 6, 2000; U.S. application Ser. No. 09/651,236, filed Aug. 29, 2000; U.S. application Ser. No. 09/636,215, filed Aug. 9, 2000; U.S. application Ser. No. 09/605,783, filed Jun. 27, 2000; U.S. application Ser. No. 09/593,793, filed Jun. 13, 2000; U.S. application Ser. No. 09/510,737, filed May 12, 2000; U.S. application Ser. No. 09/568,100, filed May 9, 2000; each a CIP of the previously filed application and pending unless noted.

----- KWIC -----

Detail Description Paragraph - DETX:

[0805] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020081609

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020081609 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------|-------|---------|---------|
| Dillon, Davin C. | Issaquah | WA | US | |
| Day, Craig H. | Shoreline | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| McNeill, Patricia D. | Federal Way | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |

APPL-NO: 09/ 910689

DATE FILED: July 20, 2001

RELATED-US-APPL-DATA:

child 09910689 A1 20010720 parent continuation-in-part-of 09778320 20010206 US
PENDING child 09778320 20010206 US parent continuation-in-part-of 09571025
20000515 US ABANDONED child 09571025 20000515 US parent continuation-in-part-of
09545068 20000407 US PENDING child 09545068 20000407 US parent
continuation-in-part-of 09523586 20000310 US ABANDONED child 09523586 20000310
US parent continuation-in-part-of 09510662 20000222 US PENDING child 09510662
20000222 US parent continuation-in-part-of 09451651 19991130 US PENDING

US-CL-CURRENT: 435/6,435/183 ,435/320.1 ,435/325 ,435/69.1 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application

Ser. No. 09/778,320, filed Feb. 6, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/571,025, filed May 15, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/545,068, filed Apr. 7, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/523,586, filed Mar. 10, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/510,662, filed Feb. 22, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/451,651, filed Nov. 30, 1999 each of which applications is incorporated herein in their entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0371] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences.** In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020076721

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020076721 A1

TITLE: Compositions and methods for the therapy and diagnosis of pancreatic cancer

PUBLICATION-DATE: June 20, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|----------|-------|---------|---------|
| Pyle, Ruth A. | Seattle | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |

APPL-NO: 09/ 923779

DATE FILED: August 6, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60291201 20010515 US
non-provisional-of-provisional 60265447 20010130 US
non-provisional-of-provisional 60223130 20000807 US

US-CL-CURRENT: 435/6,435/183 ,435/320.1 ,435/325 ,435/69.3 ,435/7.23 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly pancreatic cancer, are disclosed. Illustrative compositions comprise one or more pancreatic tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly pancreatic cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/291,201 filed May 15, 2001, U.S. Provisional Application No. 60/265,447 filed Jan. 30, 2001, and U.S. Provisional Application No. 60/223,130 filed Aug. 7, 2000, incorporated in their entirety herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0303] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020072503

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020072503 A1

TITLE: Compositions and methods for the therapy and diagnosis of ovarian cancer

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------|----------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Stolk, John A. | Bothell | WA | US | |

APPL-NO: 09/ 820089

DATE FILED: March 27, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60192530 20000328 US

US-CL-CURRENT: 514/44,424/277.1 ,424/93.21

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses an ovarian tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Diagnostic methods based on detecting an ovarian tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application is related to U.S. Provisional Application No. 60/192,530, filed Mar. 28, 2000, which is incorporated herein in its entirety.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0061] Moreover, the polynucleotide sequences of the present invention can be

engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0141] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus, even from this simplistic description of an extremely complex set of reactions, it is obvious that there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, **synthetic antisense nucleotide sequences** will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020068288

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020068288 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|---------|-------|---------|---------|
| Lodes, Michael J. | Seattle | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| Mohamath, Raodoh | Seattle | WA | US | |
| Indirias, Carol Yoseph | Seattle | WA | US | |

APPL-NO: 09/ 833790

DATE FILED: April 11, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60196780 20000411 US
non-provisional-of-provisional 60213361 20000621 US
non-provisional-of-provisional 60229763 20000901 US
non-provisional-of-provisional 60230629 20000905 US
non-provisional-of-provisional 60232565 20000914 US
non-provisional-of-provisional 60257037 20001219 US
non-provisional-of-provisional 60260796 20010108 US

US-CL-CURRENT: 435/6,435/183 ,435/320.1 ,435/325 ,435/69.1 ,435/7.23 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Applications Nos. 60/196,780, filed Apr. 11, 2000; 60/213,361, filed Jun. 21, 2000; 60/229,763, filed Sep. 1, 2000; 60/230,629, filed Sep. 5, 2000; 60/232,565, filed Sep. 14, 2000; 60/257,037, filed Dec. 19, 2000; and 60/260,796, filed Jan. 8, 2001, all of which are incorporated in their entirety herein.

----- KWIC -----

Detail Description Paragraph - DETX:

[0586] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020068285

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020068285 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|------------------|-------|---------|---------|
| Frudakis, Tony N. | Sarasota | FL | US | |
| Reed, Steven G. | Bellevue | WA | US | |
| Smith, John M. | Columbia Heights | MN | US | |
| Misher, Lynda E. | Seattle | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A. W. | Bellevue | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Day, Craig H. | Seattle | WA | US | |

APPL-NO: 09/ 810936

DATE FILED: March 16, 2001

RELATED-US-APPL-DATA:

child 09810936 A1 20010316 parent continuation-in-part-of 09699295 20001026 US
PENDING child 09699295 20001026 US parent continuation-in-part-of 09590583
20000608 US PENDING child 09590583 20000608 US parent continuation-in-part-of
09577505 20000524 US PENDING child 09577505 20000524 US parent
continuation-in-part-of 09534825 20000323 US PENDING child 09534825 20000323 US
parent continuation-in-part-of 09429755 19991028 US PENDING child 09429755
19991028 US parent continuation-in-part-of 09289198 19990409 US PENDING child
09289198 19990409 US parent continuation-in-part-of 09062451 19980417 US
PENDING child 09062451 19980417 US parent continuation-in-part-of 08991789
19971211 US GRANTED parent-patent 6225054 US child 08991789 19971211 US parent
continuation-in-part-of 08838762 19970409 US ABANDONED child 08991789 19971211
US parent continuation-in-part-of 08700014 19960820 US ABANDONED child 08700014
19960820 US parent continuation-in-part-of 08585392 19960111 US ABANDONED

US-CL-CURRENT: 435/6,435/325 ,435/69.7 ,435/7.23 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that

encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/699,295, filed Oct. 26, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/590,583, filed Jun. 8, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/577,505, filed May 24, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/534,825, filed Mar. 23, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/429,755, filed Oct. 28, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/289,198, filed Apr. 9, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/062,451, filed Apr. 17, 1998, which is a continuation in part of U.S. patent application Ser. No. 08/991,789, filed Dec. 11, 1997, which is a continuation-in-part of U.S. patent application Ser. No. 08/838,762, filed Apr. 9, 1997, now abandoned, and is a continuation-in-part of U.S. patent application Ser. No. 08/700,014, filed Aug. 20, 1996, which is a continuation-in-part of U.S. patent application No. 08/585,392, filed Jan. 11, 1996, now abandoned.

----- KWIC -----

Detail Description Paragraph - DETX:

[0168] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020064872

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020064872 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|----------|-------|---------|---------|
| Jiang, Yuqui | Kent | WA | US | |
| Dillon, Davin C. | Isaaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Xu, Jiangchun | Bellevue | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Hepler, William T. | Seattle | WA | US | |

APPL-NO: 09/ 604287

DATE FILED: June 22, 2000

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09604287 A1 20000622 parent continuation-in-part-of 09590751 20000608 US UNKNOWN

US-CL-CURRENT: 435/325,424/130.1 ,424/93.1 ,435/7.1 ,514/2 ,514/44 ,530/350 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/590,751, filed Jun. 8, 2000, which is a continuation-in-part of

U.S. patent application Ser. No. 09/551,621, filed Apr. 17, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/433,836, filed on Nov. 3, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/389,681, filed on Sep. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/389,338, filed on Jun. 23, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/285,480, filed on Apr. 2, 1999, which is a continuation-in-part of U.S. application Ser. No. 09/222,575, filed Dec. 28, 1998.

----- KWIC -----

Detail Description Paragraph - DETX:

[0536] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detail Description Paragraph - DETX:

[0610] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020053095

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020053095 A1

TITLE: METHODS FOR CONTROLLING GIBBERELLIN LEVELS

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|-------------------|-------|---------|---------|
| BROWN, SHERRI M. | CHESTERFIELD | MO | US | |
| ELICH, TEDD D. | BALLWIN | MO | US | |
| HECK, GREGORY R. | CRYSTAL LAKE PARK | MO | US | |
| KISHORE, GANESH M. | ST LOUIS | MO | US | |
| LOGUSCH, EUGENE W. | CHESTERFIELD | MO | US | |
| LOGUSCH, SHERRY J. | CHESTERFIELD | MO | US | |
| PILLER, KENNETH J. | ST LOUIS | MO | US | |
| RAO, SUDABATHULA | ST LOUIS | MO | US | |
| REAM, JOEL E. | ST LOUIS | MO | US | |

APPL-NO: 09/ 371307

DATE FILED: August 10, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

US-CL-CURRENT: 800/278

ABSTRACT:

Methods and materials are disclosed for the inhibition and control of gibberellic acid levels. In particular, nucleic acid sequences of copalyl diphosphate synthase, 3-.beta. hydroxylase, and 2-oxidase and additional nucleic acid sequences are disclosed. Gibberellic acid levels may be inhibited or controlled by preparation of a chimeric expression construct capable of expressing a RNA or protein product which suppresses the gibberellin biosynthetic pathway sequence, diverts substrates from the pathway or degrades pathway substrates or products. The sequence is preferably a copalyl diphosphate synthase sequence, a 3.beta.-hydroxylase sequence, a 2-oxidase sequence, a phytoene synthase sequence, a C20-oxidase sequence, and a 2.beta.,3.beta.-hydroxylase sequence. Administration of a complementing agent, preferably a gibberellin or gibberellin precursor or intermediate restores bioactivity.

CROSS REFERENCES TO RELATED APPLICATIONS

[0001] This Patent Application is related to U.S. Provisional Patent

Application Nos. 60/096,111, filed Aug. 10, 1998 and 60/137,977, filed Jun. 7, 1999. Both of these priority documents are incorporated by reference in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0220] The term "**gene**" refers to **chromosomal DNA, plasmid DNA, cDNA, synthetic DNA**, or other DNA that encodes a peptide, polypeptide, protein, or RNA molecule, and regions flanking the coding sequence involved in the regulation of expression.

Detail Description Paragraph - DETX:

[0262] A vector or construct may also include a transit peptide. Incorporation of a suitable chloroplast transit peptide may also be employed (European Patent Application Publication No. 0218571). The vector may also include translational enhancers. DNA constructs could contain one or more 5' non-translated leader sequences which may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic **genes, or from a synthetic gene** sequence. For a review of optimizing expression of transgenes, see Koziel et al. (Plant Mol. Biol. 32: 393-405, 1996).

Detail Description Paragraph - DETX:

[0557] A corn (Zea mays) cDNA database is searched for the GA 2-oxidase consensus sequence. Two cDNA sequences, L1892837 (SEQ ID NO:70) and L30695722 (SEQ ID NO:71), are found in leaf and 18 hour post-pollination libraries, respectively. L1892837 and L30695722 both exhibit 53% identity over 117 amino acids of At2ox1 which contains the consensus sequence found among GA 2-oxidases (SEQ ID NO:41). The methods used for cloning genomic DNA and full length cDNA clones of the Arabidopsis and soybean GA 2-oxidases as well as the methods for making plant expression vectors can be applied to clone the corn genes contained in a cDNA database of sequences contained in leaf and post-pollination cDNA libraries of corn plants. Those skilled in the art would know how to optimize expression of transgenes in monocots, such as use of introns, **codon preference**, monocot tissue and developmentally regulated **promoters**. Expression of these genes in the tissues of developing seeds, germinating seeds and during early seedling growth will result in a delay or inhibition of seed germination or reduced seedling stature. To recover seed germination and seedling height it is necessary to add exogenous bioactive gibberellic acid which is not a substrate for inactivation by GA 2-oxidases.

PGPUB-DOCUMENT-NUMBER: 20020052329

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020052329 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|------------|-------|---------|---------|
| Wang, Tongtong | Medina | WA | US | |
| Fan, Liquan | Bellevue | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| Bangur, Chaitanya S. | Seattle | WA | US | |
| Hosken, Nancy A. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A. W. | Bellevue | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| McNeill, Patricia D. | Des Moines | WA | US | |
| Fanger, Neil | Seattle | WA | US | |

APPL-NO: 09/ 735705

DATE FILED: December 12, 2000

RELATED-US-APPL-DATA:

child 09735705 A1 20001212 parent continuation-in-part-of 09685696 20001009 US
PENDING child 09735705 A1 20001212 parent continuation-in-part-of 09662786
20000915 US PENDING child 09735705 A1 20001212 parent continuation-in-part-of
09643597 20000821 US PENDING child 09735705 A1 20001212 parent
continuation-in-part-of 09630940 20000802 US PENDING child 09735705 A1 20001212
parent continuation-in-part-of 09606421 20000628 US PENDING child 09735705 A1
20001212 parent continuation-in-part-of 09542615 20000404 US PENDING child
09735705 A1 20001212 parent continuation-in-part-of 09510376 20000222 US
PENDING child 09735705 A1 20001212 parent continuation-in-part-of 09480884
20000110 US PENDING child 09735705 A1 20001212 parent continuation-in-part-of
09476496 19991230 US PENDING child 09735705 A1 20001212 parent
continuation-in-part-of 09466396 19991217 US PENDING child 09735705 A1 20001212
parent continuation-in-part-of 09285479 19990402 US PENDING child 09735705 A1
20001212 parent continuation-in-part-of 09221107 19981222 US PENDING child
09735705 A1 20001212 parent continuation-in-part-of 09123912 19980727 US
GRANTED parent-patent 6312695 US child 09735705 A1 20001212 parent
continuation-in-part-of 09040802 19980318 US PENDING

US-CL-CURRENT: 514/44,435/183 ,435/320.1 ,435/325 ,435/6 ,435/7.23 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. Patent application Ser. Nos. 09/685,696, filed Oct. 9, 2000; Ser. No. 09/662,786, filed Sep. 15, 2000; Ser. No. 09/643,597, filed Aug. 21, 2000; Ser. No. 09/630,940 filed Aug. 2, 2000; Ser. No. 09/606,421 filed Jun. 28, 2000; Ser. No. 09/542,615 filed Apr. 4, 2000; Ser. No. 09/510,376 filed Feb. 22, 2000; Ser. No. 09/480,884 filed Jan. 10, 2000; Ser. No. 09/476,496 filed Dec. 30, 1999; Ser. No. 09/466,396 filed Dec. 17, 1999; Ser. No. 09/285,479 filed Apr. 2, 1999; Ser. No. 09/221,107 filed Dec. 22, 1998; Ser. No. 09/123,912 filed Jul. 27, 1998; Ser. No. 09/040,802 filed Mar. 18, 1998; each a CIP of the previous application and pending, and all incorporated by reference herein.

----- KWIC -----

Detail Description Paragraph - DETX:

[0488] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020051977

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020051977 A1

TITLE: Compositions and methods for the therapy and diagnosis of prostate cancer

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Stolk, John A. | Bothell | WA | US | |
| Day, Craig H. | Seattle | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A. W. | Bellevue | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |
| Hural, John | Bainbridge Island | WA | US | |
| McNeill, Patricia D. | Des Moines | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |

APPL-NO: 09/ 780669

DATE FILED: February 9, 2001

RELATED-US-APPL-DATA:

child 09780669 A1 20010209 parent continuation-in-part-of 09759143 20010112 US
PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09709729
20001109 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of
09685166 20001010 US PENDING child 09780669 A1 20010209 parent
continuation-in-part-of 09679426 20001002 US PENDING child 09780669 A1 20010209
parent continuation-in-part-of 09657279 20000906 US PENDING child 09780669 A1
20010209 parent continuation-in-part-of 09651236 20000829 US PENDING child
09780669 A1 20010209 parent continuation-in-part-of 09636215 20000810 US
PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09605783
20000627 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of
09593793 20000613 US PENDING child 09780669 A1 20010209 parent

continuation-in-part-of 09510737 20000501 US GRANTED parent-patent 6219981 US child 09780669 A1 20010209 parent continuation-in-part-of 09568100 20000509 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09536857 20000327 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09483672 20000114 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09443686 19991118 US ABANDONED child 09780669 A1 20010209 parent continuation-in-part-of 09439313 19991112 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09352616 19990713 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09288946 19990409 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09232149 19990115 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09159812 19980923 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09115453 19980714 US PENDING child 09780669 A1 20010209 parent continuation-in-part-of 09030607 19980225 US GRANTED parent-patent 6262245 US child 09780669 A1 20010209 parent continuation-in-part-of 09020956 19980209 US GRANTED parent-patent 6261562 US child 09780669 A1 20010209 parent continuation-in-part-of 08904804 19970801 US ABANDONED child 09780669 A1 20010209 parent continuation-in-part-of 08806099 19970225 US ABANDONED child 09780669 A1 20010209 parent continuation-in-part-of PCT/US98/03492 19980225 US UNKNOWN child 09780669 A1 20010209 parent continuation-in-part-of PCT/US99/15838 19990714 US UNKNOWN

US-CL-CURRENT: 435/6,424/184.1 ,424/93.21 ,435/69.3 ,435/7.23 ,530/350

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. patent application Ser. No. 09/759,143, filed Jan. 12, 2001, U.S. patent application Ser. No. 09/709,729, filed Nov. 9, 2000, U.S. patent application Ser. No. 09/685,166, filed Oct. 10, 2000, U.S. patent application Ser. No. 09/679,426, filed Oct. 2, 2000, U.S. patent application Ser. No. 09/657,279, filed Sep. 6, 2000; U.S. application Ser. No. 09/651,236, filed Aug. 29, 2000; U.S. application Ser. No. 09/636,215, filed Aug. 9, 2000; U.S. application Ser. No. 09/605,783, filed Jun. 27, 2000; U.S. application Ser. No. 09/593,793, filed Jun. 13, 2000; U.S. application Ser. No. 09/510,737, filed May 12, 2000; U.S. application Ser. No. 09/568,100, filed May 9, 2000; U.S. application Ser. No. 09/536,857, filed Mar. 27, 2000; U.S. application Ser. No. 09/483,672, filed Jan. 14, 2000; U.S. application Ser. No. 09/443,686, filed Nov. 18, 1999; U.S. application Ser. No. 09/439,313, filed Nov. 12, 1999; U.S. application Ser. No. 09/352,616, filed Jul. 13, 1999; U.S. application Ser. No. 09/288,946, filed Apr. 9, 1999; U.S. application Ser. No.

09/232,149, filed Jan. 15, 1999; U.S. application Ser. No. 09/159,812, filed Sep. 23, 1998; U.S. application Ser. No. 09/115,453, filed Jul. 14, 1998; U.S. application Ser. No. 09/030,607, filed Feb. 25, 1998; U.S. application Ser. No. 09/020,956, filed Feb. 9, 1998; U.S. application Ser. No. 08/904,804, filed Aug. 1, 1997 (abandoned); U.S. application Ser. No. 08/806,099, filed Feb. 25, 1997 (abandoned); each a CIP of the previously filed application and pending unless noted; and PCT/U.S.98/03492, filed Feb. 25, 1998 (converted) and PCT/U.S.99/15838, filed Jul. 14, 1999, pending.

----- KWIC -----

Detail Description Paragraph - DETX:

[0779] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020048759

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020048759 A1

TITLE: Compositions and methods for the therapy and diagnosis of ovarian and endometrial cancer

PUBLICATION-DATE: April 25, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------|----------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Pyle, Ruth A. | Seattle | WA | US | |
| Stolk, John A. | Bothell | WA | US | |

APPL-NO: 09/ 813358

DATE FILED: March 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60190710 20000321 US
non-provisional-of-provisional 60213748 20000622 US
non-provisional-of-provisional 60257276 20001219 US

US-CL-CURRENT: 435/6,424/155.1 ,424/93.21 ,435/325 ,435/69.1 ,435/7.23 ,514/12 ,514/44 ,536/23.5

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as ovarian or endometrial cancer, are disclosed. Compositions may comprise one or more ovarian carcinoma proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses such an antigen, or a T cell that is specific for cells expressing such an antigen. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian and endometrial cancer. Diagnostic methods based on detecting an ovarian carcinoma protein, or mRNA encoding such an antigen, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Applications 60/190,710, filed Mar. 21, 2000; 60/213,748, filed Jun. 22, 2000; and 60/257,276, filed Dec. 19, 2000, all incorporated in their entirety herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0247] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0331] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, **synthetic antisense nucleotide sequences** will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020040127

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020040127 A1

TITLE: Compositions and methods for the therapy and diagnosis of colon cancer

PUBLICATION-DATE: April 4, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|----------|-------|---------|---------|
| Jiang, Yuqiu | Kent | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Clapper, Jonathan D. | Seattle | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Secrist, Heather | Seattle | WA | US | |

APPL-NO: 09/ 878722

DATE FILED: June 8, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60256571 20001218 US
non-provisional-of-provisional 60210821 20000609 US
non-provisional-of-provisional 60290240 20010510 US

US-CL-CURRENT: 530/350,435/320.1 ,435/325 ,435/69.1 ,536/23.5

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Applications 60/256,571 filed Dec. 18, 2000, 60/210,821, filed Jun. 9, 2000, and 60/290,240, filed May 10, 2001, incorporated by reference in their entirety herein.

----- KWIC -----

Detail Description Paragraph - DETX:

[0395] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020022591

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020022591 A1

TITLE: Compositions and methods for the therapy and diagnosis of ovarian cancer

PUBLICATION-DATE: February 21, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------|----------|-------|---------|---------|
| Algate, Paul A. | Issaquah | WA | US | |
| Mannion, Jane | Edmonds | WA | US | |

APPL-NO: 09/ 777564

DATE FILED: February 5, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60180403 20000204 US
non-provisional-of-provisional 60192745 20000328 US

US-CL-CURRENT: 514/12,435/6 ,435/69.1 ,436/6 ,514/44 ,530/350 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses an ovarian tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Diagnostic methods based on detecting an ovarian tumor protein, or mRNA encoding such a protein, in a sample are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/180,403, filed Feb. 4, 2000, and U.S. Provisional Application No. 60/192,745, filed Mar. 28, 2000, and are incorporated in their entirety herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0065] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Summary of Invention Paragraph - BSTX:

[0150] The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus, even from this simplistic description of an extremely complex set of reactions, it is obvious that there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

PGPUB-DOCUMENT-NUMBER: 20020022248

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020022248 A1

TITLE: Compositions and methods for the therapy and diagnosis of prostate cancer

PUBLICATION-DATE: February 21, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Dillon, Davin C. | Issaquah | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Harlocker, Susan L. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Kalos, Michael D. | Seattle | WA | US | |
| Fanger, Gary R. | Mill Creek | WA | US | |
| Retter, Marc W. | Carnation | WA | US | |
| Stolk, John A. | Bothell | WA | US | |
| Day, Craig H. | Seattle | WA | US | |
| Vedvick, Thomas S. | Federal Way | WA | US | |
| Carter, Darrick | Seattle | WA | US | |
| Li, Samuel X. | Redmond | WA | US | |
| Wang, Aijun | Issaquah | WA | US | |
| Skeiky, Yasir A. W. | Bellevue | WA | US | |
| Hepler, William T. | Seattle | WA | US | |
| Henderson, Robert A. | Edmonds | WA | US | |

APPL-NO: 09/ 759143

DATE FILED: January 12, 2001

RELATED-US-APPL-DATA:

child 09759143 A1 20010112 parent continuation-in-part-of 09685166 20001010 US
PENDING child 09759143 A1 20010112 parent continuation-in-part-of 09679426
20001002 US PENDING child 09759143 A1 20010112 parent continuation-in-part-of
09657279 20000906 US PENDING child 09759143 A1 20010112 parent
continuation-in-part-of 09651236 20000829 US PENDING child 09759143 A1 20010112
parent continuation-in-part-of 09636215 20000810 US PENDING child 09759143 A1
20010112 parent continuation-in-part-of 09605783 20000627 US PENDING child
09759143 A1 20010112 parent continuation-in-part-of 09593793 20000613 US
PENDING child 09759143 A1 20010112 parent continuation-in-part-of 09570737
20000512 US PENDING child 09759143 A1 20010112 parent continuation-in-part-of
09568100 20000509 US PENDING child 09759143 A1 20010112 parent
continuation-in-part-of 09536857 20000327 US PENDING child 09759143 A1 20010112
parent continuation-in-part-of 09483672 20000114 US PENDING child 09759143 A1
20010112 parent continuation-in-part-of 09443686 19991118 US ABANDONED child

09759143 A1 20010112 parent continuation-in-part-of 09439313 19991112 US
PENDING child 09759143 A1 20010112 parent continuation-in-part-of 09352616
19990713 US PENDING child 09759143 A1 20010112 parent continuation-in-part-of
09288946 19990409 US PENDING child 09759143 A1 20010112 parent
continuation-in-part-of 09232149 19990115 US PENDING child 09759143 A1 20010112
parent continuation-in-part-of 09159812 19980923 US PENDING child 09759143 A1
20010112 parent continuation-in-part-of 09115453 19980714 US PENDING child
09759143 A1 20010112 parent continuation-in-part-of 09030607 19980225 US
GRANTED parent-patent 6262245 US child 09759143 A1 20010112 parent
continuation-in-part-of 09020956 19980209 US GRANTED parent-patent 6261562 US
child 09759143 A1 20010112 parent continuation-in-part-of 08904804 19970801 US
ABANDONED child 09759143 A1 20010112 parent continuation-in-part-of 08806099
19970225 US ABANDONED

US-CL-CURRENT: 435/69.1,435/325 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly prostate cancer, are disclosed. Illustrative compositions comprise one or more prostate-specific polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly prostate cancer.

----- KWIC -----

Detail Description Paragraph - DETX:

[0764] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020009758

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020009758 A1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

PUBLICATION-DATE: January 24, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|---------|-------|---------|---------|
| Harlocker, Susan L. | Seattle | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| Bangur, Chaitanya S. | Seattle | WA | US | |
| Klee, Jennifer I. | Seattle | WA | US | |
| Switzer, Ann | Seattle | WA | US | |

APPL-NO: 09/ 866562

DATE FILED: May 25, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60207485 20000526 US
non-provisional-of-provisional 60230475 20000906 US

US-CL-CURRENT: 435/7.23,424/93.7 ,435/372.3 ,435/69.3 ,530/388.1 ,536/23.5

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 60/207,485, filed May 26, 2000 and U.S. Provisional Application No. 60/230,475, filed Sep. 6, 2000, incorporated in their entirety herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0244] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20020004491

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020004491 A1

TITLE: Compositions and methods for the therapy and diagnosis of ovarian cancer

PUBLICATION-DATE: January 10, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------|-------------------|-------|---------|---------|
| Xu, Jiangchun | Bellevue | WA | US | |
| Stolk, John A. | Bothell | WA | US | |
| Algate, Paul A. | Issaquah | WA | US | |
| Fling, Steven P. | Bainbridge Island | WA | US | |

APPL-NO: 09/ 825294

DATE FILED: April 3, 2001

RELATED-US-APPL-DATA:

child 09825294 A1 20010403 parent continuation-in-part-of 09713550 20001114 US
PENDING child 09713550 20001114 US parent continuation-in-part-of 09656668
20000907 US PENDING child 09656668 20000907 US parent continuation-in-part-of
09640173 20000815 US PENDING child 09640173 20000815 US parent
continuation-in-part-of 09561778 20000501 US PENDING child 09561778 20000501 US
parent continuation-in-part-of 09394374 19990910 US ABANDONED

US-CL-CURRENT: 514/44,424/155.1 ,435/183 ,435/325 ,435/69.1 ,514/12 ,530/350
,530/387.1 ,536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/713,550, filed Nov. 14, 2000, which is a CIP of Ser. No. 09/656,668, filed Sep. 7, 2000, which is a CIP of U.S. application Ser. No.

09/640,173, filed Aug. 15, 2000, which is a CIP of U.S. application Ser. No. 09/561,778, filed May 1, 2000, which is a CIP of U.S. application Ser. No. 09/394,374, filed Sep. 10, 1999, all pending and incorporated by reference in their entirety herein.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0161] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

PGPUB-DOCUMENT-NUMBER: 20010034052

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010034052 A1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

PUBLICATION-DATE: October 25, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|------------|-------|---------|---------|
| Dillon, Davin C. | Issaquah | WA | US | |
| Day, Craig H. | Seattle | WA | US | |
| Jiang, Yuqiu | Kent | WA | US | |
| Houghton, Raymond L. | Bothell | WA | US | |
| Mitcham, Jennifer L. | Redmond | WA | US | |
| Wang, Tongtong | Medina | WA | US | |
| McNeill, Patricia D. | Des Moines | WA | US | |

APPL-NO: 09/ 778320

DATE FILED: February 6, 2001

RELATED-US-APPL-DATA:

child 09778320 A1 20010206 parent continuation-in-part-of 09571025 20000515 US
PENDING child 09571025 20000515 US parent continuation-in-part-of 09545068
20000407 US PENDING child 09545068 20000407 US parent continuation-in-part-of
09523586 20000310 US PENDING child 09523586 20000310 US parent
continuation-in-part-of 09510662 20000222 US PENDING child 09510662 20000222 US
parent continuation-in-part-of 09451651 19991130 US PENDING

US-CL-CURRENT: 435/200,435/6 ,435/69.1 ,536/23.2

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly breast cancer, are disclosed. Illustrative compositions comprise one or more breast tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly breast cancer.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/571,025, filed May 15, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/545,068, filed Apr. 7, 2000, which is a

continuation-in-part of U.S. patent application Ser. No. 09/523,586, filed Mar. 10, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/510,662, filed Feb. 22, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/451,651, filed Nov. 30, 1999.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0366] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

US-PAT-NO: 6512094

DOCUMENT-IDENTIFIER: US 6512094 B1

TITLE: Compositions and methods for the therapy and diagnosis of prostate cancer

DATE-ISSUED: January 28, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|-------------|-------|----------|---------|
| Xu; Jiangchun | Bellevue | WA | N/A | N/A |
| Dillon; Davin C. | Issaquah | WA | N/A | N/A |
| Mitcham; Jennifer L. | Redmond | WA | N/A | N/A |
| Harlocker; Susan L. | Seattle | WA | N/A | N/A |
| Jiang; Yuqiu | Kent | WA | N/A | N/A |
| Kalos; Michael D. | Seattle | WA | N/A | N/A |
| Fanger; Gary R. | Mill Creek | WA | N/A | N/A |
| Retter; Marc W. | Carnation | WA | N/A | N/A |
| Stolk; John A. | Bothell | WA | N/A | N/A |
| Day; Craig H. | Seattle | WA | N/A | N/A |
| Vedvick; Thomas S. | Federal Way | WA | N/A | N/A |
| Carter; Darrick | Seattle | WA | N/A | N/A |
| Li; Samuel X. | Redmond | WA | N/A | N/A |
| Wang; Aijun | Issaquah | WA | N/A | N/A |
| Skeiky; Yasir A. W. | Bellevue | WA | N/A | N/A |
| Hepler; William T. | Seattle | WA | N/A | N/A |
| Henderson; Robert A. | Edmonds | WA | N/A | N/A |

APPL-NO: 09/ 593793

DATE FILED: June 13, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application is related to U.S. patent application Ser. No. 09/570,737, filed May 12, 2000; U.S. patent application Ser. No. 09/568,100, filed May 9, 2000; U.S. patent application Ser. No. 09/536,857, filed Mar. 27, 2000; U.S. patent application Ser. No. 09/483,672, filed Jan. 14, 2000; U.S. patent application Ser. No. 09/443,686, filed Nov. 18, 1999; U.S. patent application Ser. No. 09/439,313, filed Nov. 12, 1999; U.S. patent application Ser. No. 09/352,616, filed Jul. 13, 1999; U.S. patent application Ser. No. 09/288,946, filed Apr. 9, 1999; U.S. patent application Ser. No. 09/232,149, filed Jan. 15, 1999; U.S. patent application Ser. No. 09/159,812, filed Sep. 23, 1998; U.S. patent application Ser. No. 09/115,453, filed Jul. 14, 1998; U.S. patent application Ser. No. 09/030,607, filed Feb. 25, 1998; U.S. patent application Ser. No. 09/020,956, filed Feb. 9, 1998; U.S. patent application Ser. No. 08/904,804, filed Aug. 1, 1997, each a CIP of the previously filed application, and all pending; and U.S. patent application Ser. No. 08/806,099, filed Feb. 25, 1997, now abandoned.

US-CL-CURRENT: 530/350; 424/184.1 ; 424/185.1 ; 424/197.11 ; 435/5 ; 435/6 ; 435/91.1 ; 435/91.2 ; 530/352 ; 530/380 ; 536/23.1 ; 536/23.7

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as prostate cancer, are disclosed. Compositions may comprise one or more prostate-specific proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a prostate-specific protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as prostate cancer. Diagnostic methods based on detecting a prostate-specific protein, or mRNA encoding such a protein, in a sample are also provided.

8 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

----- KWIC -----

Detailed Description Text - DETX:

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

Detailed Description Text - DETX:

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, **synthetic antisense nucleotide sequences** will bind

to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6509448

DOCUMENT-IDENTIFIER: US 6509448 B2

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

DATE-ISSUED: January 21, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|-------------|-------|----------|---------|
| Wang; Tongtong | Medina | WA | N/A | N/A |
| Bangur; Chaitanya S. | Seattle | WA | N/A | N/A |
| Lodes; Michael J. | Seattle | WA | N/A | N/A |
| Fanger; Gary R. | Mill Creek | WA | N/A | N/A |
| Vedvick; Thomas S. | Federal Way | WA | N/A | N/A |
| Carter; Darrick | Seattle | WA | N/A | N/A |
| Retter; Marc W. | Carnation | WA | N/A | N/A |
| Mannion; Jane | Edmonds | WA | N/A | N/A |
| Fan; Liquan | Bellevue | WA | N/A | N/A |
| Wang; Aijun | Issaquah | WA | N/A | N/A |

APPL-NO: 09/ 736457

DATE FILED: December 13, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of U.S. patent application Ser. No. 09/702,705, filed Oct. 30, 2000; U.S. patent application Ser. No. 09/677,419, filed Oct. 6, 2000; U.S. patent application Ser. No. 09/671,325, filed Sep. 26, 2000; U.S. patent application Ser. No. 09/658,824, filed Sep. 8, 2000; U.S. patent application Ser. No. 09/651,563, filed Aug. 29, 2000; U.S. patent application Ser. No. 09/614,124, filed Jul. 11, 2000; U.S. patent application Ser. No. 09/589,184, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000; U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000; U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000; U.S. patent application Ser. No. 09/519,642, filed Mar. 6, 2000; U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999; U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999; U.S. patent application Ser. No. 09/419,356, filed Oct. 15, 1999; U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999 now abandoned; each a CIP of the previous application and PCT/US00/18061, filed Jun. 30, 2000, pending.

US-CL-CURRENT: 530/387.9

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer,

particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 2

----- KWIC -----

Brief Summary Text - BSTX:

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of **gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences**. In **addition, site**-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change **codon preference**, produce splice variants, or introduce mutations, and so forth.

US-PAT-NO: 6509155

DOCUMENT-IDENTIFIER: US 6509155 B1

TITLE: Nucleic acids encoding GTPase activating proteins

DATE-ISSUED: January 21, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|---------------|-------|----------|---------|
| Klinger; Tod M. | San Carlos | CA | N/A | N/A |
| Stewart; Elizabeth A. | Mountain View | CA | N/A | N/A |
| Yue; Henry | Sunnyvale | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |

APPL-NO: 09/ 507765

DATE FILED: February 18, 2000

US-CL-CURRENT: 435/6; 435/252.3 ; 435/320.1 ; 435/325 ; 435/69.1 ; 536/23.1 ; 536/23.5 ; 536/24.3

ABSTRACT:

The invention provides mammalian nucleic acid molecules and fragments thereof. It also provides for the use of the mammalian nucleic acid molecules for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with cell signaling, the immune system, and cell proliferation, particularly colon cancer. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid molecules.

13 Claims, 21 Drawing figures

Exemplary Claim Number: 3

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

"Protein" refers to an amino acid sequence, oligopeptide, peptide, polypeptide or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of nucleic acid molecules encoding GTPAP may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleic acid sequence can be engineered by such methods as DNA shuffling (Stemmer and Crameri (1996) U.S. Pat. No. 5,830,721) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources that have been selected for their efficiency in a particular host. The vector, nucleic acid molecule, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6504010

DOCUMENT-IDENTIFIER: US 6504010 B1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

DATE-ISSUED: January 7, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|-------------|-------|----------|---------|
| Wang; Tongtong | Medina | WA | N/A | N/A |
| Bangur; Chaitanya S. | Seattle | WA | N/A | N/A |
| Lodes; Michael J. | Seattle | WA | N/A | N/A |
| Fanger; Gary R. | Mill Creek | WA | N/A | N/A |
| Vedvick; Thomas S. | Federal Way | WA | N/A | N/A |
| Carter; Darrick | Seattle | WA | N/A | N/A |
| Retter; Marc W. | Carnation | WA | N/A | N/A |
| Mannion; Jane | Edmonds | WA | N/A | N/A |
| Fan; Liqun | Bellevue | WA | N/A | N/A |

APPL-NO: 09/ 702705

DATE FILED: October 30, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application is related to U.S. patent application Ser. No. 09/677,419, filed Oct. 6, 2000; U.S. patent application Ser. No. 09/671,325, filed Sep. 26, 2000; U.S. patent application Ser. No. 09/658,824, filed Sep. 8, 2000; U.S. patent application Ser. No. 09/651,563, filed Aug. 29, 2000; U.S. patent application Ser. No. 09/614,124, filed Jul. 11, 2000; U.S. patent application Ser. No. 09/589,184, filed Jun. 5, 2000; U.S. patent application Ser. No. 09/560,406, filed Apr. 27, 2000; U.S. patent application Ser. No. 09/546,259, filed Apr. 10, 2000; U.S. patent application Ser. No. 09/533,077, filed Mar. 22, 2000; U.S. patent application Ser. No. 09/519,642, filed Mar. 6, 2000; U.S. patent application Ser. No. 09/476,300, filed Dec. 30, 1999; U.S. patent application Ser. No. 09/466,867, filed Dec. 17, 1999; U.S. patent application Ser. No. 09/419,356, filed Oct. 15, 1999; U.S. patent application Ser. No. 09/346,492, filed Jun. 30, 1999 now abandoned; each a CIP of the previous application and all pending; and PCT/US00/18061, filed Jun. 30, 1999, pending.

US-CL-CURRENT: 530/350; 530/300

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, particularly lung cancer, are disclosed. Illustrative compositions comprise one or more lung tumor polypeptides, immunogenic portions thereof,

polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly lung cancer.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

US-PAT-NO: 6485919

DOCUMENT-IDENTIFIER: US 6485919 B1

TITLE: Human metabotropic glutamate receptors, nucleic acids encoding same and uses thereof

DATE-ISSUED: November 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-------------|-------|----------|---------|
| Daggett; Lorrie | San Diego | CA | N/A | N/A |
| Ellis; Steven B. | San Diego | CA | N/A | N/A |
| Liaw; Chen | San Diego | CA | N/A | N/A |
| Pontsler; Aaron | West Jordan | UT | N/A | N/A |
| Johnson; Edwin C. | San Diego | CA | N/A | N/A |
| Hess; Stephen D. | San Diego | CA | N/A | N/A |

APPL-NO: 09/ 459715

DATE FILED: December 13, 1999

PARENT-CASE:

This application is a divisional application of prior U.S. application Ser. No. 08/367,264, filed Jan. 9, 1995, and issued Dec. 14, 1999, as U.S. Pat. No. 6,001,581, which is a continuation-in-part of U.S. application Ser. No. 08/072,574, filed Jun. 4, 1993, and issued May 28, 1996, as U.S. Pat. No. 5,521,297.

US-CL-CURRENT: 435/7.21; 435/252.1 ; 435/254.2 ; 435/325 ; 435/364 ; 435/365 ; 435/366 ; 435/7.1 ; 435/7.2 ; 530/350

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human metabotropic glutamate receptor subtypes and the proteins encoded thereby. In a particular embodiment, the invention nucleic acids encode mGluR1, mGluR2, mGluR3 and mGluR5 subtypes of human metabotropic glutamate receptors. In addition to being useful for the production of metabotropic glutamate receptor subtypes, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. In addition to disclosing novel metabotropic glutamate receptor subtypes, the present invention also comprises methods for using such receptor subtypes to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function.

14 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the metabotropic glutamate receptor subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of metabotropic glutamate receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, metabotropic glutamate receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modifications may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human metabotropic glutamate receptor subtypes enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro

assay systems to determine the effects thereof.

US-PAT-NO: 6475752

DOCUMENT-IDENTIFIER: US 6475752 B1

TITLE: Mammalian imidazoline receptor

DATE-ISSUED: November 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|---------------|-------|----------|---------|
| Lal; Preeti | Santa Clara | CA | N/A | N/A |
| Tang; Y. Tom | San Jose | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |
| Kaser; Matthew R. | Castro Valley | CA | N/A | N/A |

APPL-NO: 09/ 364206

DATE FILED: July 30, 1999

US-CL-CURRENT: 435/69.1; 435/252.3 ; 435/320.1 ; 435/325 ; 435/471 ; 435/6 ; 435/70.1 ; 435/71.1 ; 435/71.2 ; 536/23.5

ABSTRACT:

The invention provide a mammalian nucleic acid molecule and fragments thereof. It also provides for the use of the mammalian nucleic acid molecule for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with its expression and for the production of a model system. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid molecule. The invention further provides a mammalian protein or portions thereof. The invention still further provides for the use of the nucleic acid molecule and protein in assays to detect or purify ligands.

12 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

"Protein" refers to an amino acid sequence, oligopeptide, peptide, polypeptide or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of nucleic acid molecules encoding mIR may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleic acid sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change **codon preference** to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (**promoters**, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid molecule, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6469142

DOCUMENT-IDENTIFIER: US 6469142 B1

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

DATE-ISSUED: October 22, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 09/ 648797

DATE FILED: August 28, 2000

PARENT-CASE:

This application is a divisional of application Ser. No. 08/940,086, filed Sep. 29, 1997, now U.S. Pat. No. 6,111,091, which is a division of application Ser. No. 08,231,193, filed Apr. 20, 1994, now U.S. Pat. No. 5,849,895, which is a continuation in part of application Ser. No. 08/052,449, filed Apr. 20, 1993, now abandoned.

US-CL-CURRENT: 530/350

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

3 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as **promoters**, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a **promoter** refers to the physical and functional relationship between the DNA and the **promoter** such that the transcription of such DNA is initiated from the **promoter** by an RNA polymerase that specifically recognizes and binds to the **promoter**, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the **codon preference** of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 **promoter**. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into **synthetic DNA sequences** which can

then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 6465200

DOCUMENT-IDENTIFIER: US 6465200 B2

TITLE: Transcription factor regulatory protein

DATE-ISSUED: October 15, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|---------------|-------|----------|---------|
| Kaser; Matthew R. | Castro Valley | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |

APPL-NO: 09/ 836941

DATE FILED: April 17, 2001

PARENT-CASE:

This application is a divisional application of U.S. application Ser. No. 09/286,132 filed Apr. 1, 1999, now U.S. Pat. No. 6,242,185, all of which application is hereby incorporated by reference herein.

US-CL-CURRENT: 435/7.8; 424/185.1 ; 530/350 ; 530/413

ABSTRACT:

The invention provides a mammalian nucleic acid sequence and fragments thereof. It also provides for the use of these nucleic acid sequences in a model system for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with expression of the mammalian nucleic acid sequence. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid sequence.

5 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

----- KWIC -----

Detailed Description Text - DETX:

"Polypeptide" refers to an amino acid, amino acid sequence, oligopeptide, peptide, or protein or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of polynucleotide sequences capable of encoding the mammalian protein may be cloned into a vector and used to express the protein, or portions thereof, in appropriate host cells. The nucleotide sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain appropriate transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and 3' untranslated regions) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid sequence, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6458561

DOCUMENT-IDENTIFIER: US 6458561 B1

TITLE: Human NIM1 kinase

DATE-ISSUED: October 1, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|---------------|-------|----------|---------|
| Bandman; Olga | Mountain View | CA | N/A | N/A |
| Molteni; Angela | Cantu | N/A | N/A | IT |
| Magnaghi; Paola | Milan | N/A | N/A | IT |
| Bosotti; Roberta | Nerviano | N/A | N/A | IT |
| Scacheri; Emanuela | Milan | N/A | N/A | IT |
| Isacchi; Antonella | Milan | N/A | N/A | IT |
| Hodgson; David M. | Palo Alto | CA | N/A | N/A |

APPL-NO: 09/ 523849

DATE FILED: March 13, 2000

US-CL-CURRENT: 435/69.1; 435/320.1 ; 435/325 ; 435/70.1 ; 536/23.1 ; 536/23.5 ; 536/24.1

ABSTRACT:

The invention provides a nucleic acid molecule which encodes the human NIM1 kinase. It also provides for the use of the nucleic acid molecule, fragments, variants and complements thereof and of the protein, portions thereof and antibodies thereto for characterization, diagnosis, evaluation, treatment, or prevention of disorders associated with expression. The invention additionally provides expression vectors and host cells for the production of the protein and a transgenic organism or model system.

12 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Detailed Description Text - DETX:

"Protein" refers to an amino acid sequence, oligopeptide, peptide, polypeptide or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of nucleic acid molecules encoding NIM1 kinase may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleic acid sequence can be engineered by such methods as DNA shuffling (U.S. Pat. No. 5,830,721) and site-directed mutagenesis t.e create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid molecule, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6432927

DOCUMENT-IDENTIFIER: US 6432927 B1

TITLE: Compositions and methods for inducing gene expression

DATE-ISSUED: August 13, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-----------|-------|----------|---------|
| Gregory; Richard J. | Westford | MA | N/A | N/A |
| Vincent; Karen | Arlington | MA | N/A | N/A |

APPL-NO: 09/ 579897

DATE FILED: May 26, 2000

PARENT-CASE:

This application claims priority to PCT patent application PCT/US98/25753, filed Dec. 4, 1998, and is a continuation-in-part of U.S. Ser. No. 09/133,612, filed Aug. 13, 1998, which claims priority under 35 U.S.C. .sectn.119(e) to provisional application No. 60/067,546, filed Dec. 4, 1997.

US-CL-CURRENT: 514/44; 424/93.2 ; 435/320.1 ; 435/325 ; 435/455 ; 435/91.4 ; 536/23.4 ; 536/24.1

ABSTRACT:

The present invention provides recombinant nucleic acid molecules encoding a chimeric transactivator protein including a DNA binding domain of a DNA binding protein and a protein domain capable of transcriptional activation. The present invention also provides recombinant viral and non-viral vectors that are able to infect and/or transfect and sustain expression of a biologically active chimeric transactivator proteins in mammalian cells. Also provided are host cell lines and non-human transgenic animals capable of expressing biologically active chimeric transactivator proteins. In another aspect, compositions and methods for treating or preventing ischemic damage associated with hypoxia-related disorders are provided.

31 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

----- KWIC -----

Detailed Description Text - DETX:

The present invention provides novel hybrid/chimeric transactivating proteins comprising a functional portion of a DNA binding protein and a functional portion of a transcriptional activator protein. The hybrid/chimeric transactivating proteins of the invention offer a variety of advantages, including the specific activation of expression of hypoxia-inducible genes containing hypoxia responsive elements (HREs), thereby achieving exceptionally high levels of gene expression. Invention hybrid/chimeric transactivating proteins are capable of functioning in vertebrate cells and may include naturally occurring transcriptional transactivating proteins or domains of proteins from eukaryotic cells including vertebrate cells, viral transactivating proteins or any **synthetic amino acid sequence** that is able to stimulate transcription from a vertebrate promoter. Examples of such transactivating proteins include, but are not limited to, the lymphoid specific transcription factor identified by Muller et al. (Nature 336:544-551 (1988)), the fos protein (Lucibello et al., Oncogene 3:43-52 (1988)); v-jun protein (Bos et al., Cell 52:705-712 (1988)); factor EF-C (Ostapchuk et al., Mol. Cell. Biol. 9:2787-2797 (1989)); HIV-1 tat protein (Arya et al., Science 229:69-73 (1985)), the papillomavirus E2 protein (Lambert et al., J. Virol. 63:3151-3154 (1989)) the adenovirus E1A protein (reviewed in Flint and Shenk, Ann. Rev. Genet. (1989), heat shock factors (HSF1 and HSF2) (Rabindran, et al., PNAS 88:6906-6910 (1991)); the p53 protein (Levine, Cell 88:323-331 (1997), Ko and Prives, Genes Dev. 10:1054-1072 (1996)); Sp1 (Kadonaga, et al. Cell 51:1079-1090 (1987)); AP1 (Lee, et al., Nature 325:368-372 (1987)); CTF/NF1 (Mermod, et al., Cell 58: 741-753 (1989)), E2F1 (Neuman, et al., Gene 173: 163-169 (1996)); HAP1 (Pfeifer, et al., Cell 56: 291-301 (1989)); HAP2 (Pinkham, et al., Mol. Cell. Biol. 7:578-585 (1987)); MCM1 (Passmore, et al., J. Mol. Biol. 204:593-606 (1988)); PHO2 (Sengstag, and Hinnen, NAR 15:233-246 (1987)); and GAL11 (Suzuki et al., Mol. Cell. Biol. 8:4991-4999 (1988)). In preferred embodiments of the invention, the transactivating protein is Herpes simplex virus VP16 (Sadowski et al., Nature 335:563-564 (1988); Triezenberg et al., Genes and Dev. 2:718-729 (1988)), NF.kappa.B ((Schmitz and Baeuerle, EMBO J. 10:3805-3817 (1991); Schmitz, et al., J.Biol.Chem. 269:25613-25620 (1994); and Schmitz, et al., J. Biol. Chem. 270:15576-15584 (1995)), and yeast activators GAL4 and GCN4.

Detailed Description Text - DETX:

Of course, the skilled artisan will understand that transcriptional activation domains useful in the compositions and methods of this invention may also be **synthetic, i.e., based on a sequence** that is not contained within a known, naturally occurring protein. See, for example, Pollock and Gilman, PNAS 94:13388-13389 (1997), which teaches that transcriptional activation is an inherently flexible process in which there is little, if any, requirement for specific structures or stereospecific protein contacts. It also reviews the variety of different molecules that can function as transcriptional activators, including short peptide motifs (as small as eight amino acids), simple amphipathic helices and even mutagenized domains of proteins unrelated to transcriptional activation.

Detailed Description Text - DETX:

Polynucleotides/transgenes are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

Detailed Description Text - DETX:

The skilled artisan will recognize that when expression from the vector is desired, the polynucleotides/transgenes are operatively linked to expression control sequences. Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the human HIF-1.alpha., EPAS1 or HLF polypeptide in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

US-PAT-NO: 6432707

DOCUMENT-IDENTIFIER: US 6432707 B1

TITLE: Compositions and methods for the therapy and diagnosis of breast cancer

DATE-ISSUED: August 13, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------|----------|-------|----------|---------|
| Reed; Steven G. | Bellevue | WA | N/A | N/A |
| Xu; Jiangchun | Bellevue | WA | N/A | N/A |
| Dillon; Davin C. | Issaquah | WA | N/A | N/A |

APPL-NO: 09/ 602877

DATE FILED: June 22, 2000

PARENT-CASE:

REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of U.S. patent application Ser. No. 09/346,327, filed Jul. 2, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/288,950, filed Apr. 9, 1999, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 09/248,178, filed Feb. 9, 1999, which is a continuation-in-part of U.S. patent application Ser. No. 09/118,627, filed Jul. 17, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/998,253, filed Dec. 24, 1997, now abandoned.

US-CL-CURRENT: 435/325; 435/252.3 ; 435/320.1 ; 514/44 ; 536/23.1

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA encoding such a protein, in a sample are also provided.

7 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX:

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Detailed Description Text - DETX:

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6426072

DOCUMENT-IDENTIFIER: US 6426072 B1

TITLE: Compositions and methods for the therapy and diagnosis of lung cancer

DATE-ISSUED: July 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|------------|-------|----------|---------|
| Wang; Tongtong | Medina | WA | N/A | N/A |
| Fan; Liqun | Bellevue | WA | N/A | N/A |
| Kalos; Michael D. | Seattle | WA | N/A | N/A |
| Bangur; Chaitanya S. | Seattle | WA | N/A | N/A |
| Hosken; Nancy A. | Seattle | WA | N/A | N/A |
| Fanger; Gary R. | Mill Creek | WA | N/A | N/A |
| Li; Samuel X. | Redmond | WA | N/A | N/A |
| Wang; Aijun | Issaquah | WA | N/A | N/A |
| Skeiky; Yasir A. W. | Bellevue | WA | N/A | N/A |
| Henderson; Robert A. | Edmonds | WA | N/A | N/A |
| McNeill; Patricia D. | Des Moines | WA | N/A | N/A |

APPL-NO: 09/ 643597

DATE FILED: August 21, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation in part of Ser. No. 09/830,940 filed Aug. 2, 2000.

US-CL-CURRENT: 424/184.1; 424/185.1 ; 435/320.1 ; 530/300 ; 530/350 ; 536/23.1 ; 536/23.4

ABSTRACT:

Compositions and methods for the therapy and diagnosis of cancer, such as lung cancer, are disclosed. Compositions may comprise one or more lung tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a lung tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as lung cancer. Diagnostic methods based on detecting a lung tumor protein, or mRNA encoding such a protein, in a sample are also provided.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

Brief Summary Text - BSTX:

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

US-PAT-NO: 6413764

DOCUMENT-IDENTIFIER: US 6413764 B1

TITLE: Human metabotropic glutamate receptors, nucleic acids encoding same and uses thereof

DATE-ISSUED: July 2, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-------------|-------|----------|---------|
| Daggett; Lorrie | San Diego | CA | N/A | N/A |
| Ellis; Steven B. | San Diego | CA | N/A | N/A |
| Liaw; Chen | San Diego | CA | N/A | N/A |
| Pontsler; Aaron | West Jordan | UT | N/A | N/A |
| Johnson; Edwin C. | San Diego | CA | N/A | N/A |
| Hess; Stephen D. | San Diego | CA | N/A | N/A |

APPL-NO: 09/ 153757

DATE FILED: September 15, 1998

PARENT-CASE:

This application is a continuation application of U.S. Ser. No. 08/486,270, now U.S. Pat. No. 5,807,689, filed Jun. 6, 1995, which is divisional application of U.S. Ser. No. 08/367,264, now U.S. Pat. No. 6,001,581, filed Jan. 9, 1995, which is a 371 of International application PCT/US94/06273, filed Jun. 3, 1994, which is in turn a continuation-in-part application of U.S. Ser. No. 08/072,574, filed Jun. 4, 1993, now U.S. Pat. No. 5,521,297, the entire contents of which are hereby incorporated by reference.

US-CL-CURRENT: 435/252.3; 435/252.8 ; 435/254.2 ; 435/254.21 ; 435/254.22 ; 435/254.3 ; 435/320.1 ; 435/325 ; 536/23.1

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human metabotropic glutamate receptor subtypes and the proteins encoded thereby. In a particular embodiment, the invention nucleic acids encode mGluR1, mGluR2, mGluR3 and mGluR5 subtypes of human metabotropic glutamate receptors. In addition to being useful for the production of metabotropic glutamate receptor subtypes, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. In addition to disclosing novel metabotropic glutamate receptor subtypes, the present invention also comprises methods for using such receptor subtypes to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function.

43 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the metabotropic glutamate receptor subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G--C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of metabotropic glutamate receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, metabotropic glutamate receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modifications may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human metabotropic glutamate receptor subtypes enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states

becomes possible, by specifically introducing such mutations into **synthetic DNA sequences** which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 6376660

DOCUMENT-IDENTIFIER: US 6376660 B1

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

DATE-ISSUED: April 23, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 935105

DATE FILED: September 29, 1997

PARENT-CASE:

This is a divisional of U.S. application Ser. No. 08/231,193, filed Apr. 20, 1994, now U.S. Pat. No. 5,849,895, which is a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now abandoned.

US-CL-CURRENT: 536/23.5; 435/252.3 ; 435/320.1 ; 435/69.1

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

21 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 6362316

DOCUMENT-IDENTIFIER: US 6362316 B1

TITLE: Human metabotropic glutamate receptor subtype mGluR6 protein

DATE-ISSUED: March 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 09/ 277858

DATE FILED: March 26, 1999

PARENT-CASE:

This application is a divisional of application Ser. No. 08/407,875, filed Mar. 20, 1995, now U.S. Pat. No. 5,912,122, which is a continuation-in-part of application Ser. No. 08/072,572, filed Jun. 4, 1993, now issued as U.S. Pat. No. 5,521,297 on May 28, 1996; the entire contents of each of which are hereby incorporated by reference herein.

US-CL-CURRENT: 530/350; 435/252.3 ; 435/254.11 ; 435/320.1 ; 435/325 ; 435/471 ; 435/69.1 ; 435/71.1 ; 435/71.2 ; 536/23.5

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human metabotropic glutamate receptor subtype mGluR6, and the proteins encoded thereby. In addition to being useful for the production of metabotropic glutamate receptor subtype mGluR6, nucleic acids of the invention are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. In addition to disclosing a novel metabotropic glutamate receptor subtype, mGluR6, the present invention also comprises methods for using the invention receptor subtype to identify and characterize compounds which affect the function of such receptor subtype, e.g., agonists, antagonists, and modulators of glutamate receptor function.

8 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as **promoters**, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a **promoter** refers to the physical and functional relationship between the DNA and the **promoter** such that the transcription of such DNA is initiated from the **promoter** by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the metabotropic glutamate receptor subunits in order to enhance transcription (e.g., the **codon preference** of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of metabotropic glutamate receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, metabotropic glutamate receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 **promoter**. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modifications may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human metabotropic glutamate receptor subtypes enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into **synthetic DNA sequences** which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 6322977

DOCUMENT-IDENTIFIER: US 6322977 B1

TITLE: Tapasin-like protein

DATE-ISSUED: November 27, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|---------------|-------|----------|---------|
| Lal; Preeti | Santa Clara | CA | N/A | N/A |
| Kaser; Matthew R. | Castro Valley | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |

APPL-NO: 09/ 292097

DATE FILED: April 14, 1999

US-CL-CURRENT: 435/6; 435/69.1 ; 530/324 ; 530/325 ; 530/350 ; 536/23.1 ; 536/24.3

ABSTRACT:

The invention provides a mammalian nucleic acid sequence and fragments thereof. It also provides for the use of the nucleic acid sequence for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with gene expression and for the production of a model system. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid sequence.

8 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Detailed Description Text - DETX:

"Protein" refers to an amino acid, amino acid sequence, oligopeptide, peptide, or polypeptide or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of nucleic acid sequences capable of encoding the mammalian protein

may be cloned into a vector and used to express the protein, or portions thereof, in appropriate host cells. The nucleotide sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain appropriate transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and 3' untranslated regions) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid sequence, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra ch. 4, 8, 16 and 17).

US-PAT-NO: 6316611

DOCUMENT-IDENTIFIER: US 6316611 B1

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

DATE-ISSUED: November 13, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 940035

DATE FILED: September 29, 1997

PARENT-CASE:

This application is a divisional of U.S. application Ser. No. 08/231,193, filed Apr. 20, 1994 which is now U.S. Pat. No. 5,849,895, a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now abandoned.

US-CL-CURRENT: 536/23.5; 435/252.3 ; 435/320.1 ; 435/69.1

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

23 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro assay systems to

determine the effects thereof.

US-PAT-NO: 6284456

DOCUMENT-IDENTIFIER: US 6284456 B1

TITLE: Transcriptional coactivator that interacts with Tat protein and regulates its binding to TAR RNA, methods for modulating Tat transactivation, and uses therefor

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|----------------|-------|----------|---------|
| Jones; Katherine A. | Encinitas | CA | N/A | N/A |
| Wei; Ping | San Diego | CA | N/A | N/A |
| Garber; Mitchell | Woodland Hills | CA | N/A | N/A |
| Fang; Shi-Min | San Diego | CA | N/A | N/A |

APPL-NO: 09/ 476482

DATE FILED: December 30, 1999

PARENT-CASE:

RELATED APPLICATIONS This application claims priority from U.S. Provisional Application No. 60/069,341, filed Dec. 11, 1997, and Continuation-in-Part application Ser. No. 09/126,980, filed Jul. 30, 1998, now pending, each of which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 435/5; 530/324 ; 530/350 ; 530/388.35 ; 536/23.1

ABSTRACT:

In accordance with the present invention, isolated nucleic acid encoding a host cell protein that regulates Tat transactivation has been discovered. The protein is the first discovered constituent of the TAK/TEFb complex which associates with the HIV Tat, via divalent cation metals, and is necessary for the binding of Tat to TAR RNA. This protein, cyclin T1, is an 87 kDa cyclin partner for the PITALRE kinase. It is further discovered that Tat must interact with TAK in order to bind to TAR RNA with affinity and with the appropriate sequence specificity that is observed in vivo. In accordance with another aspect of the invention, formulations useful for modulation of Tat transactivation have been developed. In addition, assays have been developed for the identification of compounds useful to modulate the above-described processes.

46 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Drawing Description Text - DRTX:

Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the cyclin T1 polypeptide in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Drawing Description Text - DRTX:

Also provided are vectors comprising invention nucleic acids. Examples of vectors are viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA.

US-PAT-NO: 6274331

DOCUMENT-IDENTIFIER: US 6274331 B1

TITLE: Method of determining a functional linker for fusing globin subunits

DATE-ISSUED: August 14, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Looker; Douglas L. | Lafayette | CO | N/A | N/A |
| Stetler; Gary L. | Denver | CO | N/A | N/A |

APPL-NO: 08/ 444915

DATE FILED: May 19, 1995

PARENT-CASE:

This is a division of application Ser. No. 07/789,179 filed Nov. 8, 1991, now U.S. Pat. No. 5,545,727, which is a CIP of 07/671,707 filed Apr. 1, 1991, now abandoned, which is a CIP of PCT/US90/02654 filed May 10, 1990, now abandoned, which is a CIP of (a) 07/374,161 filed Jun. 30, 1989, now abandoned, (b) 07/379,116 filed Jul. 13, 1989, now abandoned, and (c) 07/349,623 filed May 10, 1989, now abandoned, all hereby incorporated by reference herein.

US-CL-CURRENT: 435/7.2; 435/30 ; 435/39 ; 435/69.6 ; 435/69.7 ; 436/66 ; 436/86 ; 530/385

ABSTRACT:

A functional linker for a polypeptide in which two alpha or beta globin-like domains are genetically fused is determined by screening a library of genetically fused polypeptides, in which the linker region is varied, for the ability to participate in the formation of hemoglobin-like protein, as measured by the protein's response to carbon monoxide. In a preferred embodiment, cells expressing the protein turn red as a result of carbon monoxide pressure.

5 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 70

----- KWIC -----

Brief Summary Text - BSTX:

Saito, et al., J. Biochem., 101: 1281-88 (1987) expressed a **synthetic somatomedin C gene** in E. coli using a two cistron system. They theorized that the instability of somatomedin C, a basic polypeptide, might be overcome by complexing it with an acidic polypeptide. Thus, they constructed a two-cistron system which could express both polypeptides. The termination codon of the first cistron overlapped the initiation codon of the second cistron. The transformants accumulated Somatomedin C at high levels. However, the somatomedin C was recovered in the form of insoluble pellets (see page 1282).

Brief Summary Text - BSTX:

In one embodiment, the alpha- and beta-globin-like polypeptides are co-expressed in bacterial cells. The corresponding **genes may be included in the same synthetic** operon (i.e., driven by one promoter), or placed in separate operons with separate promoters (which may be the same or different). Preferably, expression of the alpha- and beta-globin is enhanced by placing a "ribosomal loader" sequence as hereafter described before each globin gene. This is particularly advantageous in the case of alpha globin which is more difficult to produce in quantity.

Drawing Description Text - DRTX:

FIG. 4: Oligonucleotides for construction of **synthetic FX-alpha and FX-beta globin genes** (4a to 4c). The top strand is shown 5' to 3' and the bottom strand as 3' to 5'. Areas of overlap between complementary synthetic oligonucleotides are shown as areas where both strands are shown in the same case letters. The PstI site that joins FX-alpha and FX-beta occurs at the overlap of SJH I-35a and SJH I-36b.

Drawing Description Text - DRTX:

FIG. 5: **Synthetic gene** for expression of Met-FX-alpha and Met-FX-beta globin (5a to 5c). Region A contains the alpha globin gene and region B the beta globin gene. The location of the Factor X sequence and the two Shine-Delgamo sequences (SD#1 and SD#2) in both regions is indicated. Selected restriction sites are also found. The translated amino acid sequences for the ribosomal loader and Met-FX-alpha/and beta-globin are given.

Drawing Description Text - DRTX:

FIG. 12 Shows the **sequence of a preferred synthetic gene** for expression of (des-Val)-alpha-(GlyGly)-alpha and des-Val beta globin (12a to 12c). A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of

mature alpha globin, and continues with the second residue, leucine. The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual alpha (or di-alpha) and beta (or di-beta) globin chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially **synthetic gene** is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). For codon preferences in yeast, see the next section. Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the **promoter** associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the **codon preference** pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong **promoter** constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

The **synthetic FX-beta gene** sequence (included in FIG. 5) was constructed as follows: 100 pmole of the following oligo nucleotides were kinased in 3 separate reactions. Reaction 1 contained oligonucleotides SJH I-36b, c, d, e, and f. Reaction 2 contained SJH I-37a, b, c, and e. Reaction 3 contained SJH I-37d, f, and SJH I-38a. After combining the appropriate oligonucleotides, the solutions were lyophilized to dryness and resuspended in 16 uL of H.sub.2O. Two uL of 10.times. kinase buffer (0.5M Tris-HCl, pH7.4, 0.1M MgCl.sub.2), 0.5 uL of 100 mM DTT, and 1 uL of 1.0 mM ATP were then added. The reaction was initiated by addition of 1 uL (2U) of T4 polynucleotide kinase (IBI, Inc., New Haven, Conn.). After incubation at 37.degree. C. for 1 hour, the reactions were heated to 95.degree. C. for 10 minutes to inactivate the kinase. The three reactions were combined and 100 pmoles of oligonucleotides SJH I-36a and SJH I-38b were added. After addition of 10 uL of 100 mM Tris, pH 7.8, 100 mM

MgCl₂.sub.2, the oligonucleotides were allowed to anneal by incubating at 65.degree. C. for 30 min, 37.degree. C. for 30 min, and 15.degree. C. for 1 hour. Annealed oligonucleotides were ligated by addition of ATP (1 mM, final) and DTT (10 mM final) and 4 uL (20U) T4 DNA ligase (IBI, Inc., New Haven, Conn.) and incubation at 15.degree. C. for 1 hour. Aliquots of this ligation mixture were then cloned directly into M13mp19 (see below).

Detailed Description Text - DETX:

Other hemoglobin mutants: The synthetic genes encoding Hemoglobin Cheverly (beta.sup.45 phe.fwdarw.ser) Hemoglobin Providence/MSR (beta.sup.82 lys.fwdarw.asp) and Hemoglobin beta.sup.67 val.fwdarw.ile and Hemoglobin Kansas (beta .sup.102 asn.fwdarw.thr) were prepared similarly except with synthetic oligonucleotides spanning the SacII.fwdarw.BglII, Sall .fwdarw.SpeI, NcoI.fwdarw.KpnI and SacI.fwdarw.Sel restriction sites respectively (FIG. 7). Synthesis of the mutant oligonucleotides, restriction enzyme digestion, gel purification, and ligation conditions were identical to those used for Hemoglobin Beth Israel. All mutations were first cloned into plasmid pDL II-10a, appropriate clones were sequenced, and the mutated beta globin gene was subcloned into PstI and HindIII digested pDL II-66a. Plasmid sequencing was accomplished as described previously. E. coli cells were transformed, cultured, and induced as previously described. FX-hemoglobin mutants were purified by the method of Example 3. Oxygen binding of purified hemoglobin mutants is shown in Table 9.

Detailed Description Text - DETX:

The recognition site (FX)-encoding sequence could now be removed from pGEM FX-alpha and pGEM FX-beta to obtain pDL II-91f and pDL II-95a, respectively. The des-val alpha globin gene of pDL II-91f was recloned into pKK 223-3 to generate pDL III-1a, the gene being operably linked to the Tac promoter of pKK-223-3. The des-val beta globin gene of pDL II-95a was purified and inserted downstream of the des-val alpha globin gene of pDL III-1a to form a single transcriptional unit which would encode a polycistronic alpha globin/beta globin mRNA, see pDL III-14c. Finally, a synthetic oligonucleotide comprising the desired di-alpha linker encoding sequence and another copy of the alpha globin gene was inserted into pDL III-14c to create pDL III-47a, wherein a Tac promoter controls transcription of a di-alpha globin gene and a des-val beta globin gene.

Detailed Description Text - DETX:

The EagI and PstI restriction fragment containing most of the alpha globin gene from the plasmid pDL II-91f was gel purified and ligated to a synthetic DNA linker containing the sequence from the BstBI site of the alpha globin gene to the codon (wild-type Arginine) for its carboxyl terminus, a variable glycine-encoding linker (for example, FIG. 12, RGGV, a di-glycine followed by .alpha. Val; other possibilities include RGM, RGV, RGGV, etc., See Table 200), and the codons for the amino terminal region of alpha globin to the EagI site (FIG. 12). After digesting this ligation mixture with Pst I, the resulting

fragment was cloned into BstBI/PstI-cut pDL III-14C to create plasmid pDL III-47a (RGM-di-alpha). Plasmids pDL III-82a (RGGV-di-alpha), pDL IV-8a (RGV-di-alpha), pDL IV-976 (RV-di-alpha) and pDL IV-66a (RGGGV-di-alpha) were similarly constructed to incorporate the indicated changes in the di-alpha coding sequences.

Detailed Description Text - DETX:

Prior to inserting the globin **genes into the vector it was necessary to incorporate the synthetic translational coupler sequence** into the HpaI site of pPL-lambda-E. This was done by digestion of pPL-lambda-E with HpaI followed by blunt-end ligation of the co-translational coupler into the HpaI site of the vector. Ligation of the coupler to the blunt end resulted in destruction of the HpaI site. The ligation mixture was treated with HpaI to digest any plasmid remaining containing the HpaI site. E. coli N99Ci+ cells were transformed with the resulting reaction mixture. Clones were screened with EcoRI and Hind III restriction digests to identify clones containing the co-translational coupler in the proper orientation. DNA fragments of 522 bp and 4762 bp were observed for plasmid containing the desired orientation. To confirm the orientation of the coupler, the resulting plasmid was sequenced using a primer (5'CAATGGAAAGCAGCAAATCC-3') complementary to the sequence 30 base pairs upstream from the translational coupler sequence. The desired plasmid was denoted as pPL-lambda-E+TC.

Detailed Description Text - DETX:

This **synthetic promoter consists of two functional parts, a regulatory sequence** and sequence that allows efficient initiation of mRNA synthesis. One of the regulatory regions we chose includes the nucleotide sequence that confers positive regulation of transcription in the presence of galactose (M. Johnston and R. Davis, 1984. Molecular and Cellular Biology 4:1440-1448; L. Guarente et al., 1982, Proc Nat Acad Sci (USA) 79:7410-7414.). The transcriptional initiation site is derived from the consensus sequence for the S.cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene (GAP491) (L. McAlister and M. J. Holland, J. Biol Chem 260:15019-15027, 1983; J. P. Holland et al., J. Biol Chem 258:5291-5299, 1983).

Detailed Description Text - DETX:

ASSEMBLY OF THE **SYNTHETIC GALACTOSE UPSTREAM ACTIVATOR (GAL.sub.UAS) SEQUENCE**

Detailed Description Text - DETX:

The next step in the assembly of this hybrid promoter was to clone the SphI-SalI fragment containing the GAL.sub.UAS into pGS2888. pGS2888 was digested with SphI and SalI, phenol-chloroform extracted and ethanol precipitated. Fifty nanograms of SphI, SalI digested pGS2888 was incubated with 25 ng of the annealed, ligated GAL.sub.UAS mixture in 0.005 ml 1.times.

ligase buffer containing 10 units of T4 DNA ligase. The ligation mixture was incubated overnight at 4.degree. C. and a portion used to transform E.coli DH5.alpha.. Ampicillin resistant clones were isolated and plasmid DNA prepared. The plasmid DNA (digested with XbaI and SphI) was analyzed by agarose gel electrophoresis. A plasmid containing a fragment of the expected size (.sup..about. 500 bp) was identified. The sequence of the putative GAL.sub.UAS portion of this plasmid was determined and the plasmid was designated pGS4788 (FIG. 21(b)). The complete sequence of the synthetic GALGAP promotor (pGGAP) is shown in FIG. 20.

Detailed Description Text - DETX:

Removal of the PstI and SphI sites from pGS4888. The design of the synthetic linker for joining two .alpha.-globin chains allows the inclusion of PstI and SphI sites flanking a 30 bp sequence that includes the junction of the two .alpha.-globin coding sequences. Because we anticipate testing several different linker sequences, these sites will allow directional cloning of relatively short synthetic oligonucleotides encoding different linker sequences. Removal of the PstI and SphI sites from the vector sequence is, therefore, necessary so that the sites in the coding region are usable. One .mu.g of the plasmid pGS4888 was digested with PstI and ethanol precipitated. The dry pellet was resuspended in 50 .mu.l of 33 mM Tris-acetate, pH7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT and 50 .mu.M of each dNTP (T4 polymerase buffer). Two units of T4 DNA polymerase were added and the reaction mixture incubated for 15 min at 37.degree. C. Na.sub.3 EDTA was added to 12.5 mM and the reaction mixture heated to 65.degree. C. for 15 min, phenol extracted and ethanol precipitated. The dry pellet was dissolved in 14 .mu.l of T4 DNA ligase buffer (BRL) and 1 .mu.l (10 units) of DNA ligase added. The ligation mixture was incubated a 4.degree. C. for 16 hr. A portion of the ligation reaction was used to transform E.coli DH5.alpha. and transformants were selected on LB-ampicillin plates. Plasmid DNA was prepared from 12 transformants. The DNA was analyzed by agarose gel electrophoresis of PstI digests. Five transformants had lost the PstI site and one of these was designated pGS1889. The SphI site of this plasmid was removed as described above after digestion of pGS1889 with SphI. A plasmid was identified that had lost both the PstI and the SphI site and was designated pGS1989.

Detailed Description Text - DETX:

Parental plasmid is pSGE224 (42) The linker sequences are synthetic DNA.

Other Reference Publication - OREF:

Schulz et al., "Increased Expression in E. coli of a Synthetic Gene . . . " J. Bacteriology 169(12): 5385-5392, Dec. 1987.

US-PAT-NO: 6270956

DOCUMENT-IDENTIFIER: US 6270956 B1

TITLE: Transcriptional coactivator that interacts with Tat protein and regulates its binding to TAR RNA, methods for modulating Tat transactivation, and uses therefor

DATE-ISSUED: August 7, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|----------------|-------|----------|---------|
| Jones; Katherine A. | Encinitas | CA | N/A | N/A |
| Wei; Ping | Brookeville | MD | N/A | N/A |
| Garber; Mitchell | Woodland Hills | CA | N/A | N/A |
| Fang; Shi-Min | San Diego | CA | N/A | N/A |

APPL-NO: 09/ 126980

DATE FILED: July 30, 1998

PARENT-CASE:

RELATED APPLICATIONS This application claims priority from U.S. Provisional Application Ser. No. 60/069,341, filed Dec. 11, 1997, now abandoned, which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 435/5; 530/324 ; 530/350 ; 530/388.35

ABSTRACT:

In accordance with the present invention, a host cell protein has been discovered which regulates Tat transactivation. The protein is the first discovered constituent of the TAK/TEFb complex which associates with the HIV Tat, via divalent cation metals, and is necessary for the binding of Tat to TAR RNA. This protein, cyclin T1, is an 87 kDa cyclin partner for the PITALRE kinase. It is further discovered that Tat must interact with TAK in order to bind to TAR RNA with affinity and with the appropriate sequence specificity that is observed in vivo. In accordance with another aspect of the invention, formulations useful for modulation of Tat transactivation have been developed. In addition, assays have been developed for the identification of compounds useful to modulate the above-described processes.

20 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Detailed Description Text - DETX:

Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the cyclin T1 polypeptide in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Also provided are vectors comprising invention nucleic acids. Examples of vectors are viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA.

US-PAT-NO: 6245526

DOCUMENT-IDENTIFIER: US 6245526 B1

TITLE: Lipid metabolism transcription factor

DATE-ISSUED: June 12, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|---------------|-------|----------|---------|
| Yue; Henry | Sunnyvale | CA | N/A | N/A |
| Kaser; Matthew R. | Castro Valley | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |

APPL-NO: 09/ 318978

DATE FILED: May 26, 1999

US-CL-CURRENT: 435/69.1; 435/252.3 ; 435/320.1 ; 435/325 ; 536/23.1

ABSTRACT:

The invention provides a mammalian nucleic acid sequence and fragments thereof. It also provides for the use of these nucleic acid sequences in a model system for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with expression of the mammalian nucleic acid sequence. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid sequence.

13 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

----- KWIC -----

Detailed Description Text - DETX:

"Polypeptide" refers to an amino acid, amino acid sequence, oligopeptide, peptide, or protein or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of polynucleotide sequences capable of encoding the mammalian

protein may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleotide sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and 3' untranslated regions) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid sequence, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6242185

DOCUMENT-IDENTIFIER: US 6242185 B1

TITLE: Purified nucleic acid encoding transcription factor regulatory protein

DATE-ISSUED: June 5, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|---------------|-------|----------|---------|
| Kaser; Matthew R. | Castro Valley | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |

APPL-NO: 09/ 286132

DATE FILED: April 1, 1999

US-CL-CURRENT: 435/6; 435/243 ; 435/320.1 ; 435/325 ; 435/410 ; 435/69.1 ; 536/23.5

ABSTRACT:

The invention provides a mammalian nucleic acid sequence and fragments thereof. It also provides for the use of these nucleic acid sequences in a model system for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with expression of the mammalian nucleic acid sequence. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid sequence.

12 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

----- KWIC -----

Detailed Description Text - DETX:

"Polypeptide" refers to an amino acid, amino acid sequence, oligopeptide, peptide, or protein or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of polynucleotide sequences capable of encoding the mammalian

protein may be cloned into a vector and used to express the protein, or portions thereof, in appropriate host cells. The nucleotide sequence can be engineered by such methods as DNA shuffling (Stemmer and Crameri (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change **codon preference** to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain appropriate transcriptional and translational control elements (**promoters**, enhancers, specific initiation signals, and 3' untranslated regions) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid sequence, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6184356

DOCUMENT-IDENTIFIER: US 6184356 B1

TITLE: Production and use of multimeric hemoglobins

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Anderson; David C. | San Bruno | CA | N/A | N/A |
| Mathews; Antony J. | Boulder | CO | N/A | N/A |
| Stetler; Gary L. | Boulder | CO | N/A | N/A |

APPL-NO: 09/ 058562

DATE FILED: April 13, 1998

PARENT-CASE:

This is a division of parent application Ser. No. 08/443,890 filed May 31, 1995, now U.S. Pat. No. 5,739,011, which is a continuation of Ser. No. 08/240,712 filed May 9, 1994, now U.S. Pat. No. 5,599,907, which is a continuation-in-part of Ser. No. 07/789,179 filed Nov. 8, 1991, now U.S. Pat. No. 5,545,727, which is a continuation-in-part of Ser. No. 07/671,707 filed Apr. 1, 1991, now abandoned, which is a division of PCT/US90/02654 filed May 10, 1990, which is a continuation-in-part of (a) Ser. No. 07/374,161 filed Jun. 30, 1989, now abandoned, (b) Ser. No. 07/379,116 filed Jul. 13, 1989, now abandoned, and (c) Ser. No. 07/349,623 filed May 10, 1989, now abandoned.

US-CL-CURRENT: 530/385; 530/400 ; 530/402 ; 530/417

ABSTRACT:

DNA molecules which encode pseudodimeric globin-like polypeptides with an asymmetric cysteine mutation suitable for crosslinking two tetramers, or which encode pseudooligomeric globin-like polypeptides comprising four or more globin-like domains, are useful in the preparation of multimeric hemoglobin-like proteins.

7 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Drawing Description Text - DRTX:

FIGS. 2a-2e Shows the sequence [SEQ ID NO:1] of a preferred synthetic gene for expression of (des-Val)-alpha-(Gly)-alpha and des-Val beta globin. This gene is carried by pSGE1.1E4. A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) (SEQ ID NO:23) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The residues are numbered 1 to 141 (SEQ ID NO:24). The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. The residues are numbered 1' to 141' (SEQ ID NO:25). Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. The beta residues are numbered 1 to 146. (SEQ ID NO:26). A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual polypeptide chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially synthetic gene is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the promoter associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the codon preference pattern of the gene. When secretion of the gene- product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong promoter constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

This is a derivative of pKK223-3 (Pharmacia LKB, Piscataway, N.J., USA) and pGEM1 (Promega Corp., Madison, Wis., USA) which carries synthetic genes for des-Val alpha globin and des-Val beta globin as part of a polycistronic operon

driven by a single Tac promoter.

Other Reference Publication - OREF:

Schoner, B. et al/Expression of Eukaryotic **Genes in Escherichia coli with a Synthetic** Two Cistron System/Methods in Enzymology/vol. 153 Recombinant DNA Part D/ed: Ray Wu & L Grossman/Academic Press, Inc./NY/(1987), 401-416.

US-PAT-NO: 6168933

DOCUMENT-IDENTIFIER: US 6168933 B1

TITLE: Phospholipid transfer protein

DATE-ISSUED: January 2, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|---------------|-------|----------|---------|
| Kaser; Matthew R. | Castro Valley | CA | N/A | N/A |
| Hillman; Jennifer L. | Mountain View | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |

APPL-NO: 09/ 328869

DATE FILED: June 8, 1999

US-CL-CURRENT: 435/91.1; 435/6 ; 435/69.1 ; 435/91.2 ; 436/94 ; 536/23.1

ABSTRACT:

The invention provides a mammalian nucleic acid molecule and fragments thereof. It also provides for the use of the nucleic acid molecule for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with gene expression and for the production of a model system. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid molecule.

13 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

----- KWIC -----

Detailed Description Text - DETX:

"Protein" refers to an amino acid, amino acid sequence, oligopeptide, peptide, or polypeptide or portions thereof whether naturally occurring or synthetic.

Detailed Description Text - DETX:

A multitude of nucleic acid molecules capable of encoding the mammalian protein may be cloned into a vector and used to express the protein, or portions

thereof, in host cells. The nucleotide sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change **codon preference** to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (**promoters**, enhancers, specific initiation signals, and 3' untranslated regions) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid sequence, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6166180

DOCUMENT-IDENTIFIER: US 6166180 A

TITLE: Chromosome 21 gene marker, compositions and methods using same

DATE-ISSUED: December 26, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-------------|-------|----------|---------|
| Korenberg; Julie R. | Los Angeles | CA | N/A | N/A |
| Yamakawa; Kazuhiro | Los Angeles | CA | N/A | N/A |

APPL-NO: 09/ 048887

DATE FILED: March 26, 1998

PARENT-CASE:

This application is a divisional of application Ser. No. 08/377,690, filed Nov. 9, 1994 U.S. Pat. No. 5,773,268.

US-CL-CURRENT: 530/350

ABSTRACT:

The present invention provides isolated nucleic acids encoding human EHOC-1 protein and isolated receptor proteins encoded thereby. Further provided are vectors containing invention nucleic acids, probes that hybridize thereto, host cells transformed therewith, antisense oligonucleotides thereto and compositions containing, antibodies that specifically bind to invention polypeptides and compositions containing, as well as transgenic non-human mammals that express the invention protein.

6 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Detailed Description Text - DETX:

Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and

Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the EHOC-1 polypeptide in order to enhance transcription (e.g., the codon preference of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Also provided are vectors comprising the invention nucleic acids. Examples of vectors are viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

US-PAT-NO: 6114123

DOCUMENT-IDENTIFIER: US 6114123 A

TITLE: Lipocalin family protein

DATE-ISSUED: September 5, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|----------------|-------|----------|---------|
| Murry; Lynn E. | Portola Valley | CA | N/A | N/A |
| Tang; Tom Y. | San Jose | CA | N/A | N/A |
| Baughn; Mariah R. | San Leandro | CA | N/A | N/A |

APPL-NO: 09/ 332934

DATE FILED: June 14, 1999

US-CL-CURRENT: 435/6; 435/252.3 ; 435/320.1 ; 435/325 ; 435/69.1 ; 530/300 ; 530/350 ; 536/23.1

ABSTRACT:

The invention provide a mammalian nucleic acid molecule and fragments thereof. It also provides for the use of the mammalian nucleic acid molecule for the characterization, diagnosis, evaluation, treatment, or prevention of conditions, diseases and disorders associated with gene expression and for the production of a model system. The invention additionally provides expression vectors and host cells for the production of the protein encoded by the mammalian nucleic acid molecule.

13 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

"Protein" refers to an amino acid **sequence, oligopeptide, peptide, polypeptide or portions thereof whether naturally occurring or synthetic.**

Detailed Description Text - DETX:

A multitude of nucleic acid molecules encoding the mammalian lipocalin family

protein may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleic acid sequence can be engineered by such methods as DNA shuffling (Stemmer and Cramer (1996) U.S. Pat. No. 5,830,721 incorporated by reference herein) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources which have been selected for their efficiency in a particular host. The vector, nucleic acid molecule, and regulatory elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

US-PAT-NO: 6111091

DOCUMENT-IDENTIFIER: US 6111091 A

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefore

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 940086

DATE FILED: September 29, 1997

PARENT-CASE:

This application is a divisional application of U.S. Ser. No. 08/231,193, filed Apr. 20, 1994, now issued on Dec. 15, 1998 as U.S. Pat. No. 5,849,895, and a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now abandoned.

US-CL-CURRENT: 435/325; 435/320.1 ; 435/69.1 ; 536/23.1

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

12 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro assay systems to

determine the effects thereof.

US-PAT-NO: 6100046

DOCUMENT-IDENTIFIER: US 6100046 A

TITLE: Methods of identifying modulators of alpha9, a novel
acetylcholine-gated ion channel receptor subunit

DATE-ISSUED: August 8, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------------|-------------|-------|----------|---------|
| Elgoyhen; Ana Belen | Del Mar | CA | N/A | N/A |
| Johnson; David S. | La Jolla | CA | N/A | N/A |
| Boulter; James Richard | Los Angeles | CA | N/A | N/A |
| Heinemann; Stephen Fox | La Jolla | CA | N/A | N/A |

APPL-NO: 08/ 471961

DATE FILED: June 5, 1995

PARENT-CASE:

RELATED INVENTIONS This application is a divisional of U.S. application Ser. No. 08/278,635, filed Jul. 21, 1994, now U.S. Pat. No. 5,683,912, which is a continuation-in-part of U.S. Application Ser. No. 07/898,185, filed Jun. 12, 1992, now U.S. Pat. No. 5,371,188, which is a continuation of U.S. Application Ser. No. 07/664,473, filed Mar. 4, 1991, which is a continuation of U.S. application Ser. No. 07/321,374, filed Mar. 10, 1989, now U.S. Pat. No. 4,899,689, which is a continuation-in-part of U.S. application Ser. No. 07/170,295, filed Mar. 18, 1988, now abandoned.

US-CL-CURRENT: 435/7.2; 435/6 ; 435/7.1 ; 436/501

ABSTRACT:

The present invention provides isolated nucleic acids encoding alpha9 nicotinic acetylcholine receptor subunit and receptor subunit protein encoded thereby. Also provided are vectors containing the invention nucleic acids, host cells transformed therewith, alpha9 nicotinic acetylcholine receptor subunit and functional nicotinic acetylcholine receptors comprising at least one alpha9 subunit expressed recombinantly in such host cells as well as transgenic non-human mammals that express the invention receptor subunit and mutants thereof. Receptors of the invention comprise at least one alpha9 nicotinic acetylcholine subunit and form cationic channels activated by acetylcholine, but blocked by nicotine and muscarine. The invention also provides methods for identifying compounds that modulate the ion channel activity of the functional invention receptors containing at least one invention subunit.

18 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

----- KWIC -----

Detailed Description Text - DETX:

Vectors employed in the present invention contain both a **promoter** and a cloning site into which nucleic acid encoding alpha9 receptor subunit(s) can be operatively linked. Such vectors, which are well known in the art, are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation.

Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for native codons of the alpha9 nAChR subunit in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Examples of suitable vectors that may be employed in the present invention include viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Invention nucleic acids are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic linkers can be ligated to the termini of restricted invention nucleic acids. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA. Additionally, a nucleic acid containing a termination codon and an appropriate restriction site can be ligated into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

US-PAT-NO: 6030806

DOCUMENT-IDENTIFIER: US 6030806 A

TITLE: Human chromosome 16 genes, compositions, methods of making and using same

DATE-ISSUED: February 29, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|--------------|-------|----------|---------|
| Landes; Gregory M. | Northborough | MA | 01522 | N/A |
| Burn; Timothy C. | Northborough | MA | 01522 | N/A |
| Connors; Timothy D. | Hopkinton | MA | 01748 | N/A |
| Dackowski; William R. | Hopkinton | MA | 01748 | N/A |
| Van Raay; Terence J. | Hudson | MA | 01749 | N/A |
| Klinger; Katherine W. | Sudbury | MA | 01776 | N/A |

APPL-NO: 08/ 762500

DATE FILED: December 9, 1996

PARENT-CASE:

This application is a continuation-in-part of U.S. application Ser. No. 08/665,259, filed Jun. 17, 1996, currently pending, which is a continuation-in-part of U.S. application Ser. No. 60/000,596, filed Jun. 30, 1995.

US-CL-CURRENT: 435/69.1; 435/320.1 ; 435/325 ; 435/375 ; 435/6 ; 435/70.1 ; 435/71.1 ; 536/23.5 ; 536/24.31 ; 536/24.33 ; 800/13 ; 800/14 ; 800/21 ; 800/9

ABSTRACT:

In accordance with the present invention, there are provided isolated nucleic acids encoding a human netrin, a human ATP binding cassette transporter, a human ribosomal L3 subtype, and a human augments of liver regeneration as well as isolated protein products encoded thereby. The present invention provides nucleic acid probes that hybridize to invention nucleic acids as well as isolated nucleic acids comprising unique gene sequences located on chromosome 16. Further provided are vectors containing invention nucleic acids, host cells transformed therewith, as well as transgenic non-human mammals that express invention polypeptides. The present invention includes antisense oligonucleotides, antibodies and compositions containing same. Additionally, the invention provides methods for identifying compounds that bind to invention polypeptides.

25 Claims, 48 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 50

----- KWIC -----

Detailed Description Text - DETX:

Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the human netrin, human ABC3 transporter, the human ribosomal L3 subtype, or the human augmentin of liver regeneration polypeptide in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

US-PAT-NO: 6028173

DOCUMENT-IDENTIFIER: US 6028173 A

TITLE: Human chromosome 16 genes, compositions, methods of making and using same

DATE-ISSUED: February 22, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|--------------|-------|----------|---------|
| Landes; Gregory | Northborough | MA | N/A | N/A |
| Burn; Timothy | Northborough | MA | N/A | N/A |
| Connors; Timothy | Hopkinton | MA | N/A | N/A |
| Dackowski; William | Hopkinton | MA | N/A | N/A |
| Van Raay; Terence | Hudson | MA | N/A | N/A |
| Klinger; Katherine | Sudbury | MA | N/A | N/A |

APPL-NO: 08/ 665259

DATE FILED: June 17, 1996

PARENT-CASE:

This application claims benefit of provisional Appln 60/000,596 filed Jun. 30, 1995.

US-CL-CURRENT: 530/350; 530/300 ; 530/325 ; 530/364

ABSTRACT:

This invention provides an isolated human netrin polypeptide. Netrins define a family of diffusable factors involved in axon outgrowth.

6 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 29

----- KWIC -----

Detailed Description Text - DETX:

Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and

Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the human netrin, human ABC3 transporter, the human ribosomal L3 subtype, or the human augmentor of liver regeneration polypeptide in order to enhance transcription (e.g., the codon preference of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColEI for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

US-PAT-NO: 6013766

DOCUMENT-IDENTIFIER: US 6013766 A

TITLE: Cloning and expression of a novel acetylcholine-gated ion channel receptor subunit

DATE-ISSUED: January 11, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------------|-----------|-------|----------|---------|
| Elgoyhen; Ana Belen | Del Mar | CA | N/A | N/A |
| Johnson; David S. | La Jolla | CA | N/A | N/A |
| Boulter; James Richard | San Diego | CA | N/A | N/A |
| Heinemann; Stephen Fox | La Jolla | CA | N/A | N/A |

APPL-NO: 08/ 464258

DATE FILED: June 5, 1995

PARENT-CASE:

RELATED INVENTIONS This application is a divisional of application Ser. No. 08/278,635, filed Jul. 21, 1994, now U.S. Pat. No. 5,683,912, which is a continuation-in-part of application Ser. No. 07/898,185, filed Jun. 12, 1992, now U.S. Pat. No. 5,371,188, which is a continuation of application Ser. No. 07/664,473, filed Mar. 4, 1991, now abandoned, which is a continuation of application Ser. No. 07/321,374, filed Mar. 14, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07/170,295, filed Mar. 18, 1988, now abandoned.

US-CL-CURRENT: 530/350

ABSTRACT:

The present invention provides isolated nucleic acids encoding alpha9 nicotinic acetylcholine receptor subunit and receptor subunit protein encoded thereby. Also provided are vectors containing the invention nucleic acids, host cells transformed therewith, alpha9 nicotinic acetylcholine receptor subunit and functional nicotinic acetylcholine receptors comprising at least one alpha9 subunit expressed recombinantly in such host cells as well as transgenic non-human mammals that express the invention receptor subunit and mutants thereof. Receptors of the invention comprise at least one alpha9 nicotinic acetylcholine subunit and form cationic channels activated by acetylcholine, but blocked by nicotine and muscarine. The invention also provides methods for identifying compounds that modulate the ion channel activity of the functional invention receptors containing at least one invention subunit.

4 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Detailed Description Text - DETX:

Vectors employed in the present invention contain both a **promoter** and a cloning site into which nucleic acid encoding alpha9 receptor subunit(s) can be operatively linked. Such vectors, which are well known in the art, are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation.

Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for native codons of the alpha9 nAChR subunit in order to enhance transcription (e.g., the **codon preference** of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Examples of suitable vectors that may be employed in the present invention include viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Invention nucleic acids are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic linkers can be ligated to the termini of restricted invention nucleic acids. These **synthetic linkers contain nucleic acid sequences** that correspond to a particular restriction site in the vector DNA. Additionally, a nucleic acid containing a termination codon and an appropriate restriction site can be ligated into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

US-PAT-NO: 6001581

DOCUMENT-IDENTIFIER: US 6001581 A

TITLE: Cation-based bioassay using human metabotropic glutamate receptors

DATE-ISSUED: December 14, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-----------|-------|----------|---------|
| Johnson; Edwin C. | San Diego | CA | N/A | N/A |
| Hess; Stephen D. | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 367264

DATE FILED: January 9, 1995

PARENT-CASE:

RELATED APPLICATIONS This application is a 371 of International application PCT/US94/06273, filed June 3, 1994, which is in turn a continuation-in-part application of U.S. Application Ser. No. 08/072,574, filed June 4, 1993, now U.S. Pat. No. 5,521,297, the entire contents of which are hereby incorporated by reference.

PCT-DATA:

APPL-NO: PCT/US94/06273
DATE-FILED: June 3, 1994
PUB-NO: WO94/29449
PUB-DATE: Dec 22, 1994
371-DATE: Jan 9, 1995
102(E)-DATE: Jan 9, 1995

US-CL-CURRENT: 435/7.21; 435/325 ; 435/69.1 ; 436/501 ; 530/350 ; 536/23.5

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human metabotropic glutamate receptor subtypes and the proteins encoded thereby. In a particular embodiment, the invention nucleic acids encode mGluR1, mGluR2, mGluR3 and mGluR5 subtypes of human metabotropic glutamate receptors. In addition to being useful for the production of metabotropic glutamate receptor subtypes, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. In addition to disclosing novel metabotropic glutamate receptor subtypes, the present invention also comprises methods for using such receptor subtypes to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function.

15 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the metabotropic glutamate receptor subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of metabotropic glutamate receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, metabotropic glutamate receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modifications may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human metabotropic glutamate receptor subtypes enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states

becomes possible, by specifically introducing such mutations into **synthetic DNA sequences** which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 5985586

DOCUMENT-IDENTIFIER: US 5985586 A

TITLE: Methods for identifying compounds that modulate the activity of human N-methyl-D-aspartate receptors

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Ellis; Steven B. | San Diego | CA | N/A | N/A |
| Liaw; Chen Wang | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 486273

DATE FILED: June 6, 1995

PARENT-CASE:

This application is a divisional of U.S. Ser. No. 08/231,193, filed Apr. 20, 1994, now U.S. Pat. No. 5,849,895, which is a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now abandoned, the entire contents of which are herein incorporated by reference.

US-CL-CURRENT: 435/7.21; 435/254.11 ; 435/325 ; 435/69.1 ; 435/7.1 ; 435/7.2 ; 530/350 ; 536/23.5

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises

methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

4 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the

creation of animal models of such disease states becomes possible, by specifically introducing such mutations into **synthetic DNA sequences** which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 5912122

DOCUMENT-IDENTIFIER: US 5912122 A

TITLE: Nucleic acids encoding and method for detecting nucleic acid encoding human metabotropic glutamate receptor subtype mGluR6

DATE-ISSUED: June 15, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 407875

DATE FILED: March 20, 1995

PARENT-CASE:

The present application is a continuation-in-part of U.S. Ser. No. 08/072,574, filed Jun. 4, 1993, now U.S. Pat. No. 5,521,297.

US-CL-CURRENT: 435/6; 435/325 ; 536/23.5 ; 536/24.31

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human metabotropic glutamate receptor subtype mGluR6, and the proteins encoded thereby. In addition to being useful for the production of metabotropic glutamate receptor subtype mGluR6, nucleic acids of the invention are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. In addition to disclosing a novel metabotropic glutamate receptor subtype, mGluR6, the present invention also comprises methods for using the invention receptor subtype to identify and characterize compounds which affect the function of such receptor subtype, e.g., agonists, antagonists, and modulators of glutamate receptor function.

12 Claims, 1 Drawing figures

Exemplary Claim Number: 1,6,11

Number of Drawing Sheets: 1

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the metabotropic glutamate receptor subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of metabotropic glutamate receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and .sub.3' untranslated sequences, respectively. For example, metabotropic glutamate receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070) a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modifications may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human metabotropic glutamate receptor subtypes enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 5849895

DOCUMENT-IDENTIFIER: US 5849895 A

TITLE: Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor

DATE-ISSUED: December 15, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------|-------|----------|---------|
| Daggett; Lorrie P. | San Diego | CA | N/A | N/A |
| Lu; Chin-Chun | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 231193

DATE FILED: April 20, 1994

PARENT-CASE:

This application is a division of U.S. Ser. No. 08/231,193, filed Apr. 20, 1994, and a continuation-in-part of U.S. Ser. No. 08/052,449, filed Apr. 20, 1993, now abandoned.

US-CL-CURRENT: 536/23.5; 435/252.3 ; 435/320.1 ; 435/69.1

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human NMDA receptor protein subunits and the proteins encoded thereby. The NMDA receptor subunits of the invention comprise components of NMDA receptors that have cation-selective channels and bind glutamate and NMDA. In one aspect of the invention, the nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. In a preferred embodiment, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits of human NMDA receptors. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of one type of NMDA receptor subunit protein (homomeric) or from a mixture of two or more types of subunit proteins (heteromeric). In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

25 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the .beta.-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro assay systems to determine the effects thereof.

US-PAT-NO: 5844089

DOCUMENT-IDENTIFIER: US 5844089 A

TITLE: Genetically fused globin-like polypeptides having hemoglobin-like activity

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|------------|-------|----------|---------|
| Hoffman; Stephen J. | Denver | CO | N/A | N/A |
| Looker; Douglas L. | Lafayette | CO | N/A | N/A |
| Rosendahl; Mary S. | Broomfield | CO | N/A | N/A |
| Stetler; Gary L. | Denver | CO | N/A | N/A |
| Wagenbach; Michael | Osaka | N/A | N/A | JP |
| Anderson; David C. | Lafayette | CO | N/A | N/A |
| Mathews; Antony James | Louisville | CO | N/A | N/A |
| Nagai; Kiyoshi | Cambridge | N/A | N/A | GB2 |

APPL-NO: 08/ 450733

DATE FILED: May 25, 1995

PARENT-CASE:

This is a division of application Ser. No. 07/789,179 filed Nov. 8, 1991, now U.S. Pat. No. 5,545,727, which is a CIP of Ser. No. 07/671,707 filed Apr. 1, 1991, now abandoned, which is a CIP of PCT/US90/02654 filed May 10, 1990, now abandoned, which is a CIP of (a) Ser. No. 07/374,161 filed Jun. 30, 1989, now abandoned, (b) Ser. No. 07/379,116 filed Jul. 13, 1989, now abandoned, and (c) Ser. No. 07/349,623 filed May 10, 1989, now abandoned, all hereby incorporated by reference herein. CROSS REFERENCE TO RELATED APPLICATIONS Hoffman and Nagai, U.S. Ser. No. 07/194,338, filed May 10, 1988, now U.S. Pat. No. 5,028,588, presently owned by Somatogen, Inc., relates to the use of low oxygen affinity and other mutant hemoglobins as blood substitutes, and to the expression of alpha and beta globin in nonerythroid cells. Hoffman and Nagai, U.S. Ser. No. 07/443,950, filed Dec. 1, 1989, discloses certain additional dicysteine hemoglobin mutants; it is a continuation-in-part of 07/194,338. Anderson, et al., HEMOGLOBINS AS DRUG DELIVERY AGENTS Atty. Docket.: ANDERSON5-USA, filed Nov. 8, 1991, discloses use of conjugation of hemoglobins with drugs as a means for delivery of the drug to a patient.

US-CL-CURRENT: 530/385

ABSTRACT:

The alpha subunits of hemoglobin, which in nature are formed as separate polypeptide chains which bind noncovalently to the beta subunits, are here provided in the form of the novel molecule di-alpha globin, a single

polypeptide chain defined by connecting the two alpha subunits either directly via peptide bond or indirectly by a flexible amino acid or peptide linker. Di-alpha globin may be combined in vivo or in vitro with beta globin and heme to form hemoglobin. Di-alpha globin is expressed by recombinant DNA techniques. Di-beta globin may be similarly obtained.

23 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 70

----- KWIC -----

Brief Summary Text - BSTX:

Saito, et al., J. Biochem., 101: 1281-88 (1987) expressed a **synthetic somatomedin C gene** in E. coli using a two cistron system. They theorized that the instability of somatomedin C, a basic polypeptide, might be overcome by complexing it with an acidic polypeptide. Thus, they constructed a two-cistron system which could express both polypeptides. The termination codon of the first cistron overlapped the initiation codon of the second cistron. The transformants accumulated Somatomedin C at high levels. However, the somatomedin C was recovered in the form of insoluble pellets (see page 1282).

Brief Summary Text - BSTX:

In one embodiment, the alpha- and beta-globin-like polypeptides are co-expressed in bacterial cells. The corresponding **genes may be included in the same synthetic** operon (i.e., driven by one promoter), or placed in separate operons with separate promoters (which may be the same or different). Preferably, expression of the alpha- and beta-globin is enhanced by placing a "ribosomal loader" sequence as hereafter described before each globin gene. This is particularly advantageous in the case of alpha globin which is more difficult to produce in quantity.

Drawing Description Text - DRTX:

FIG. 4: oligonucleotides for construction of **synthetic FX-alpha and FX-beta globin genes** (4a to 4c). The top strand is shown 5' to 3' and the bottom strand as 3' to 5'. Areas of overlap between complementary synthetic oligonucleotides are shown as areas where both strands are shown in the same case letters. The PstI site that joins FX-alpha and FX-beta occurs at the overlap of SJH I-35a and SJH I-36b.

Drawing Description Text - DRTX:

FIG. 5: **Synthetic gene** for expression of Met-FX-alpha and Met-FX-beta globin

(5a to 5c). Region A contains the alpha globin gene and region B the beta globin gene. The location of the Factor X sequence and the two Shine-Delgamo sequences (SD#1 and SD#2) in both regions is indicated. Selected restriction sites are also found. The translated amino acid sequences for the ribosomal loader and Met-FX-alpha/and beta-globin are given.

Drawing Description Text - DRTX:

FIG. 12 Shows the sequence of a preferred synthetic gene for expression of (des-Val)-alpha-(GlyGly)-alpha and des-Val beta globin. A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual alpha (or di-alpha) and beta (or di-beta) globin chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially synthetic gene is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). For codon preferences in yeast, see the next section. Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the promoter associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the codon preference pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong promoter constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

The **synthetic FX-beta gene** sequence (included in FIG. 5) was constructed as follows: 100 pmole of the following oligo nucleotides were kinased in 3 separate reactions. Reaction 1 contained oligonucleotides SJH I-36b, c, d, e, and f. Reaction 2 contained SJH I-37a, b, c, and e. Reaction 3 contained SJH I-37d, f, and SJH I-38a. After combining the appropriate oligonucleotides, the solutions were lyophilized to dryness and resuspended in 16 uL of H.sub.2 O. Two uL of 10.times. kinase buffer (0.5M Tris-HCl, pH 7.4, 0.1M MgCl.sub.2), 0.5 uL of 100 mM DTT, and 1 uL of 1.0 mM ATP were then added. The reaction was initiated by addition of 1 uL(2 U) of T4 polynucleotide kinase (IBI, Inc., New Haven, Conn.). After incubation at 37.degree. C. for 1 hour, the reactions were heated to 95.degree. C. for 10 minutes to inactivate the kinase. The three reactions were combined and 100 pmoles of oligonucleotides SJH I-36a and SJH I-38b were added. After addition of 10 uL of 100 mM Tris, pH 7.8, 100 mM MgCl.sub.2, the oligonucleotides were allowed to anneal by incubating at 65.degree. C. for 30 min, 37.degree. C. for 30min, and 15.degree. C. for 1 hour. Annealed oligonucleotides were ligated by addition of ATP (1 mM, final) and DTT (10 mM final) and 4 uL (20 U) T4 DNA ligase (IBI, Inc., New Haven, Conn.) and incubation at 15.degree. C. for 1 hour. Aliquots of this ligation mixture were then cloned directly into M13mp19 (see below).

Detailed Description Text - DETX:

Other hemoglobin mutants: The **synthetic genes** encoding Hemoglobin Cheverly (beta.sup.45 phe.fwdarw.ser) Hemoglobin Providence/MSR (beta.sup.82 lys.hoarfrost.asp) and Hemoglobin beta.sup.67 val.fwdarw.ile and Hemoglobin Kansas (beta .sup.102 asn.fwdarw.thr) were prepared similarly except with synthetic oligonucleotides spanning the SacII.fwdarw.BglII, Sall.fwdarw.SpeI, NcoI.fwdarw.KpnI and SacI.fwdarw.SpeI restriction sites respectively (FIG. 7). Synthesis of the mutant oligonucleotides, restriction enzyme digestion, gel purification, and ligation conditions were identical to those used for Hemoglobin Beth Israel. All mutations were first cloned into plasmid pDL II-10a, appropriate clones were sequenced, and the mutated beta globin gene was subcloned into PstI and HindIII digested pDL II-66a. Plasmid sequencing was accomplished as described previously. E. coli cells were transformed, cultured, and induced as previously described. FX-hemoglobin mutants were purified by the method of Example 3. Oxygen binding of purified hemoglobin mutants is shown in Table 9.

Detailed Description Text - DETX:

The recognition site (FX)-encoding sequence could now be removed from pGEM FX-alpha and pGEM FX-beta to obtain pDL II-91f and pDL II-95a, respectively. The des-val alpha globin gene of pDL II-91f was recloned into pKK 223-3 to generate pDL III-1a, the gene being operably linked to the Tac promoter of pKK-223-3. The des-val beta globin gene of pDL II-95a was purified and inserted downstream of the des-val alpha globin gene of pDL III-1a to form a single transcriptional unit which would encode a polycistronic alpha globin/beta globin mRNA, see pDL III-14c. Finally, a **synthetic oligonucleotide**

comprising the desired di-alpha linker encoding sequence and another copy of the alpha globin gene was inserted into pDL III-14c to create pDL III-47a, wherein a Tac promoter controls transcription of a di-alpha globin gene and a des-val beta globin gene.

Detailed Description Text - DETX:

The EagI and PstI restriction fragment containing most of the alpha globin gene from the plasmid pDL II-91f was gel purified and ligated to a synthetic DNA linker containing the sequence from the BstBI site of the alpha globin gene to the codon (wild-type Arginine) for its carboxyl terminus, a variable glycine-encoding linker (for example, FIG. 12, RGGV, a di-glycine followed by .alpha. Val; other possibilities include RGM, RGV, RGGV, etc., See Table 200), and the codons for the amino terminal region of alpha globin to the EagI site (FIG. 12). After digesting this ligation mixture with Pst I, the resulting fragment was cloned into BstBI/PstI-cut pDL III-14C to create plasmid pDL III-47a (RGM-di-alpha). Plasmids pDL III-82a (RGGV-di-alpha), pDL IV-8a (RGV-di-alpha), pDL IV-976 (RV-di-alpha) and pDL IV-66a (RGGGV-di-alpha) were similarly constructed to incorporate the indicated changes in the di-alpha coding sequences.

Detailed Description Text - DETX:

Prior to inserting the globin genes into the vector it was necessary to incorporate the synthetic translational coupler sequence into the HpaI site of pPL-lambda-E. This was done by digestion of pPL-lambda-E with HpaI followed by blunt-end ligation of the co-translational coupler into the HpaI site of the vector. Ligation of the coupler to the blunt end resulted in destruction of the HpaI site. The ligation mixture was treated with HpaI to digest any plasmid remaining containing the HpaI site. E. coli N99Ci+ cells were transformed with the resulting reaction mixture. Clones were screened with EcoRI and Hind III restriction digests to identify clones containing the co-translational coupler in the proper orientation. DNA fragments of 522 bp and 4762 bp were observed for plasmid containing the desired orientation. To confirm the orientation of the coupler, the resulting plasmid was sequenced using a primer (5'CAATGGAAAGCAGCAAATCC-3') complementary to the sequence 30 base pairs upstream from the translational coupler sequence. The desired plasmid was denoted as pPL-lambda-E+TC.

Detailed Description Text - DETX:

This synthetic promoter consists of two functional parts, a regulatory sequence and sequence that allows efficient initiation of mRNA synthesis. One of the regulatory regions we chose includes the nucleotide sequence that confers positive regulation of transcription in the presence of galactose (M. Johnston and R. Davis, 1984. Molecular and Cellular Biology 4:1440-1448; L. Guarente et al., 1982, Proc Nat Acad Sci (USA) 79:7410-7414.). The transcriptional initiation site is derived from the consensus sequence for the S. cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene (GAP491) (L. McAlister and M. J. Holland, J. Biol Chem 260:15019-15027, 1983; J. P. Holland et al., J. Biol Chem

258:5291-5299, 1983).

Detailed Description Text - DETX:

ASSEMBLY OF THE SYNTHETIC GALACTOSE UPSTREAM ACTIVATOR (GAL.sub.UAS SEQUENCE

Detailed Description Text - DETX:

The next step in the assembly of this hybrid promoter was to clone the SphI - Sall fragment containing the GAL.sub.UAS into pGS2888. pGS2888 was digested with SphI and Sall, phenol-chloroform extracted and ethanol precipitated. Fifty nanograms of SphI, Sall digested pGS2888 was incubated with 25 ng of the annealed, ligated GAL.sub.UAS mixture in 0.005 ml 1.times. ligase buffer containing 10 units of T4 DNA ligase. The ligation mixture was incubated overnight at 4.degree. C. and a portion used to transform E. coli DH5.alpha.. Ampicillin resistant clones were isolated and plasmid DNA prepared. The plasmid DNA (digested with XbaI and SphI) was analyzed by agarose gel electrophoresis. A plasmid containing a fragment of the expected size (.sup.13 500bp) was identified. The sequence of the putative GAL.sub.UAS portion of this plasmid was determined and the plasmid was designated pGS4788 (FIG. 21(b)). The complete sequence of the synthetic GALGAP promotor (PGGAP) is shown in FIG. 20.

Detailed Description Text - DETX:

The design of the synthetic linker for joining two .alpha.-globin chains allows the inclusion of PstI and SpeI sites flanking a 30 bp sequence that includes the junction of the two .alpha.-globin coding sequences. Because we anticipate testing several different linker sequences, these sites will allow directional cloning of relatively short synthetic oligonucleotides encoding different linker sequences. Removal of the PstI and SpeI sites from the vector sequence is, therefore, necessary so that the sites in the coding region are usable. One .mu.g of the plasmid pGS4888 was digested with PstI and ethanol precipitated. The dry pellet was resuspended in 50 .mu.l of 33 mM Tris-acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT and 50 .mu.M of each dNTP (T4 polymerase buffer). Two units of T4 DNA polymerase were added and the reaction mixture incubated for 15 min at 37.degree. C. Na.sub.3 EDTA was added to 12.5 mM and the reaction mixture heated to 65.degree. C. for 15min, phenol extracted and ethanol precipitated. The dry pellet was dissolved in 14 .mu.l of T4 DNA ligase buffer (BRL) and 1 .mu.l (10 units) of DNA ligase added. The ligation mixture was incubated a 4.degree. C. for 16hr. A portion of the ligation reaction was used to transform E. coli DH5.alpha. and transformants were selected on LB-ampicillin plates. Plasmid DNA was prepared from 12 transformants. The DNA was analyzed by agarose gel electrophoresis of PstI digests. Five transformants had lost the PstI site and one of these was designated pGS1889. The SpeI site of this plasmid was removed as described above after digestion of pGS1889 with SpeI. A plasmid was identified that had lost both the PstI and the SpeI site and was designated pGS1989.

Detailed Description Text - DETX:

Parental plasmid is pSGE224 (42) The linker sequences are synthetic DNA.

US-PAT-NO: 5844088

DOCUMENT-IDENTIFIER: US 5844088 A

TITLE: Hemoglobin-like protein comprising genetically fused globin-like polypeptides

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|------------|-------|----------|---------|
| Hoffman; Stephen J. | Denver | CO | N/A | N/A |
| Looker; Douglas L. | Lafayette | CO | N/A | N/A |
| Rosendahl; Mary S. | Broomfield | CO | N/A | N/A |
| Stetler; Gary L. | Denver | CO | N/A | N/A |
| Wagenbach; Michael | Osaka | N/A | N/A | JP |
| Anderson; David C. | Lafayette | CO | N/A | N/A |
| Mathews; Antony James | Louisville | CO | N/A | N/A |
| Nagai; Kiyoshi | Cambridge | N/A | N/A | GB2 |

APPL-NO: 08/ 444991

DATE FILED: May 19, 1995

PARENT-CASE:

This is a division of application Ser. No. 07/789,179 filed Nov. 8, 1991, now U.S. Pat. No. 5,545,727, which is a CIP of Ser. No. 07/671,707 filed Apr. 1, 1991, now abandoned, which is a CIP of PCT/US90/02654 filed May 10, 1990, now abandoned, which is a CIP of (a) Ser. No. 07/374,161 filed Jun. 30, 1989, now abandoned, (b) Ser. No. 07/379,116 filed Jul. 13, 1989, now abandoned, and (c) Ser. No. 07/349,623 filed May 10, 1989, now abandoned, all hereby incorporated by reference herein.

US-CL-CURRENT: 530/385

ABSTRACT:

The alpha subunits of hemoglobin, which in nature are formed as separate polypeptide chains which bind noncovalently to the beta subunits, are here provided in the form of the novel molecule di-alpha globin, a single polypeptide chain defined by connecting the two alpha subunits either directly via peptide bond or indirectly by a flexible amino acid or peptide linker. Di-alpha globin may be combined in vivo or in vitro with beta globin and heme to form hemoglobin. Di-alpha globin is expressed by recombinant DNA techniques. Di-beta globin may be similarly obtained.

We further describe the production of tetrameric human hemoglobin and di-alpha/beta.sub.2 hemoglobin in the yeast *Saccharomyces cerevisiae*. The synthesis of the protein is directed by a synthetic promotor consisting of two

functional parts, an upstream activator sequence (UAS) that confers inducible transcription by galactose from a consensus yeast transcriptional initiation site. The expression construct is designed such that translation is expected to initiate at the same position as the human wild-type genes for .alpha.- and .beta.-globin. Three different types of expression vectors have been used: (1) .alpha.-globin and .beta.-globin contained on two separate plasmids (pGS4688 and pGS4988) in a diploid yeast strain; (2) .alpha.-globin and .beta.-globin each contained on a single plasmid (pGS289 and pGS389) and expressed in either haploid or diploid strains; and (3) di-alpha-globin and beta globin contained on a single plasmid (pGS 3089)and expressed in haploid strains.

Finally, we describe the co-expression of alpha and beta globin chains. The chains are folded together and combined intracellularly with heme to form active tetrameric hemoglobin. The hemoglobin may be recovered from the cells' soluble fraction. The invention thus obviates the need to express alpha and beta globin separately, solubilize, renature and purify them, and combine them in vitro with heme to obtain an artificial hemoglobin. By way of comparison, the separately expressed beta globin known in the art is deposited in inclusion bodies. Polycistronic co-expression of alpha and beta globins is particularly preferred.

50 Claims, 71 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 69

----- KWIC -----

Abstract Text - ABTX:

We further describe the production of tetrameric human hemoglobin and di-alpha/beta.sub.2 hemoglobin in the yeast *Saccharomyces cerevisiae*. The synthesis of the protein is directed by a **synthetic promotor consisting of two functional parts, an upstream activator sequence** (UAS) that confers inducible transcription by galactose from a consensus yeast transcriptional initiation site. The expression construct is designed such that translation is expected to initiate at the same position as the human wild-type genes for .alpha.- and .beta.-globin. Three different types of expression vectors have been used: (1) .alpha.-globin and .beta.-globin contained on two separate plasmids (pGS4688 and pGS4988) in a diploid yeast strain; (2) .alpha.-globin and .beta.-globin each contained on a single plasmid (pGS289 and pGS389) and expressed in either haploid or diploid strains; and (3) di-alpha-globin and beta globin contained on a single plasmid (pGS 3089)and expressed in haploid strains.

Brief Summary Text - BSTX:

Saito, et al., J. Biochem., 101: 1281-88 (1987) expressed a **synthetic somatomedin C gene** in *E. coli* using a two cistron system. They theorized that the instability of somatomedin C, a basic polypeptide, might be overcome by

complexing it with an acidic polypeptide. Thus, they constructed a two-cistron system which could express both polypeptides. The termination codon of the first cistron overlapped the initiation codon of the second cistron. The transformants accumulated Somatomedin C at high levels. However, the somatomedin C was recovered in the form of insoluble pellets (see page 1282).

Brief Summary Text - BSTX:

In one embodiment, the alpha- and beta-globin-like polypeptides are co-expressed in bacterial cells. The corresponding **genes may be included in the same synthetic** operon (i.e., driven by one promoter), or placed in separate operons with separate promoters (which may be the same or different). Preferably, expression of the alpha- and beta-globin is enhanced by placing a "ribosomal loader" sequence as hereafter described before each globin gene. This is particularly advantageous in the case of alpha globin which is more difficult to produce in quantity.

Drawing Description Text - DRTX:

FIG. 4: oligonucleotides for construction of **synthetic FX-alpha and FX-beta globin genes** (4a to 4c). The top strand is shown 5' to 3' and the bottom strand as 3' to 5'. Areas of overlap between complementary synthetic oligonucleotides are shown as areas where both strands are shown in the same case letters. The PstI site that joins FX-alpha and FX-beta occurs at the overlap of SJH I-35a and SJH I-36b.

Drawing Description Text - DRTX:

FIG. 5: **Synthetic gene** for expression of Met-FX-alpha and Met-FX-beta globin (5a to 5c). Region A contains the alpha globin gene and region B the beta globin gene. The location of the Factor X sequence and the two Shine-Delgarno sequences (SD#1 and SD#2) in both regions is indicated. Selected restriction sites are also found. The translated amino acid sequences for the ribosomal loader and Met-FX-alpha/and beta-globin are given.

Drawing Description Text - DRTX:

FIG. 12: Shows the **sequence of a preferred synthetic gene** for expression of (des-Val)-alpha-(GlyGly)-alpha and des-Val beta globin (12a to 12c). A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta

globin. A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual alpha (or di-alpha) and beta (or di-beta) globin chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially **synthetic gene** is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). For codon preferences in yeast, see the next section. Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the **promoter** associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the **codon preference** pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong **promoter** constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

The **synthetic FX-beta gene** sequence (included in FIG. 5) was constructed as follows: 100 pmole of the following oligonucleotides were kinased in 3 separate reactions. Reaction 1 contained oligonucleotides SJH I-36b, c, d, e, and f. Reaction 2 contained SJH I-37a, b, c, and e. Reaction 3 contained SJH I-37d, f, and SJH I-38a. After combining the appropriate oligonucleotides, the solutions were lyophilized to dryness and resuspended in 16 uL of H.sub.2 O. Two uL of 10.times. kinase buffer (0.5M Tris-HCl, pH 7.4, 0.1M MgCl.sub.2), 0.5 uL of 100 mM DTT, and 1 uL of 1.0 mM ATP were then added. The reaction was initiated by addition of 1 uL (2 U) of T4 polynucleotide kinase (IBI, Inc., New Haven, Conn.). After incubation at 37.degree. C. for 1 hour, the reactions were heated to 95.degree. C. for 10 minutes to inactivate the kinase. The three reactions were combined and 100 pmoles of oligonucleotides SJH I-36a and SJH I-38b were added. After addition of 10 uL of 100 mM Tris, pH 7.8, 100 mM MgCl.sub.2, the oligonucleotides were allowed to anneal by incubating at 65.degree. C. for 30 min, 37.degree. C. for 30 min, and 15.degree. C. for 1 hour. Annealed oligonucleotides were ligated by addition of ATP (1 mM, final) and DTT (10 mM final) and 4 uL (20 U) T4 DNA ligase (IBI, Inc., New Haven,

Conn.) and incubation at 15.degree. C. for 1 hour. Aliquots of this ligation mixture were then cloned directly into M13mp19 (see below).

Detailed Description Text - DETX:

The **synthetic genes** encoding Hemoglobin Cheverly (beta.sup.45 phe.fwdarw.ser) Hemoglobin Providence/MSR (beta.sup.82 lys.fwdarw.asp) and Hemoglobin beta.sup.67 val.fwdarw.ile and Hemoglobin Kansas (beta.sup.102 asn.fwdarw.thr) were prepared similarly except with synthetic oligonucleotides spanning the SacII.fwdarw.BglII, Sall.fwdarw.SpeI, NcoI.fwdarw.KpnI and SacI.fwdarw.SpeI restriction sites respectively (FIG. 7). Synthesis of the mutant oligonucleotides, restriction enzyme digestion, gel purification, and ligation conditions were identical to those used for Hemoglobin Beth Israel. All mutations were first cloned into plasmid pDL II-10a, appropriate clones were sequenced, and the mutated beta globin gene was subcloned into PstI and HindIII digested pDL II-66a. Plasmid sequencing was accomplished as described previously. E. coli cells were transformed, cultured, and induced as previously described. FX-hemoglobin mutants were purified by the method of Example 3. oxygen binding of purified hemoglobin mutants is shown in Table 9.

Detailed Description Text - DETX:

The recognition site (FX)-encoding sequence could now be removed from pGEM FX-alpha and pGEM FX-beta to obtain pDL II-91f and pDL II-95a, respectively. The des-val alpha globin gene of pDL II-91f was recloned into pKK 223-3 to generate pDL III-1a, the gene being operably linked to the Tac promoter of pKK-223-3. The des-val beta globin gene of pDL II-95a was purified and inserted downstream of the des-val alpha globin gene of pDL III-1a to form a single transcriptional unit which would encode a polycistronic alpha globin/beta globin mRNA, see pDL III-14c. Finally, a **synthetic oligonucleotide comprising the desired di-alpha linker encoding sequence** and another copy of the alpha globin gene was inserted into pDL III-14c to create pDL III-47a, wherein a Tac promoter controls transcription of a di-alpha globin gene and a des-val beta globin gene.

Detailed Description Text - DETX:

The EagI and PstI restriction fragment containing most of the alpha globin **gene from the plasmid pDL II-91f was gel purified and ligated to a synthetic DNA linker containing the sequence** from the BstBI site of the alpha globin gene to the codon (wild-type Arginine) for its carboxyl terminus, a variable glycine-encoding linker (for example, FIG. 12, RGGV, a di-glycine followed by .alpha. Val; other possibilities include RGM, RGV, RGGV, etc., See Table 200), and the codons for the amino terminal region of alpha globin to the EagI site (FIG. 12). After digesting this ligation mixture with Pst I, the resulting fragment was cloned into BstBI/PstI-cut pDL III-14C to create plasmid pDL III-47a (RGM-di-alpha). Plasmids pDL III-82a (RGGV-di-alpha), pDL IV-8a (RGV-di-alpha), pDL IV-976 (RV-di-alpha) and pDL IV-66a (RGGGV-di-alpha) were similarly constructed to incorporate the indicated changes in the di-alpha coding sequences.

Detailed Description Text - DETX:

Prior to inserting the globin genes into the vector it was necessary to incorporate the synthetic translational coupler sequence into the HpaI site of pPL-lambda-E. This was done by digestion of pPL-lambda-E with HpaI followed by blunt-end ligation of the co-translational coupler into the HpaI site of the vector. Ligation of the coupler to the blunt end resulted in destruction of the HpaI site. The ligation mixture was treated with HpaI to digest any plasmid remaining containing the HpaI site. E. coli N99Ci+ cells were transformed with the resulting reaction mixture. Clones were screened with EcoRI and Hind III restriction digests to identify clones containing the co-translational coupler in the proper orientation. DNA fragments of 522 bp and 4762 bp were observed for plasmid containing the desired orientation. To confirm the orientation of the coupler, the resulting plasmid was sequenced using a primer (5'CAATGGAAAGCAGCAAATCC-3') complementary to the sequence 30 base pairs upstream from the translational coupler sequence. The desired plasmid was denoted as pPL-lambda-E+TC.

Detailed Description Text - DETX:

This synthetic promoter consists of two functional parts, a regulatory sequence and sequence that allows efficient initiation of mRNA synthesis. One of the regulatory regions we chose includes the nucleotide sequence that confers positive regulation of transcription in the presence of galactose (M. Johnston and R. Davis, 1984. Molecular and Cellular Biology 4:1440-1448; L. Guarente et al., 1982, Proc Nat Acad Sci (U.S.A.) 79:7410-7414.). The transcriptional initiation site is derived from the consensus sequence for the S. cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene (GAP491) (L. McAlister and M. J. Holland, J. Biol Chem 260:15019-15027, 1983; J. P. Holland et al., J. Biol Chem 258:5291-5299, 1983).

Detailed Description Text - DETX:

ASSEMBLY OF THE SYNTHETIC GALACTOSE UPSTREAM ACTIVATOR (GAL.sub.UAS) SEQUENCE

Detailed Description Text - DETX:

The next step in the assembly of this hybrid promoter was to clone the SphI-Sall fragment containing the GAL.sub.UAS into pGS2888. pGS2888 was digested with SphI and Sall, phenol-chloroform extracted and ethanol precipitated. Fifty nanograms of SphI, Sall digested pGS2888 was incubated with 25ng of the annealed, ligated GAL.sub.UAS mixture in 0.005 ml 1.times. ligase buffer containing 10 units of T4 DNA ligase. The ligation mixture was incubated overnight at 4.degree. C. and a portion used to transform E. coli DH5.alpha.. Ampicillin resistant clones were isolated and plasmid DNA prepared. The plasmid DNA (digested with XbaI and SphI) was analyzed by agarose gel electrophoresis. A plasmid containing a fragment of the expected size

(.sup..about. 500 bp) was identified. The sequence of the putative GAL.sub.UAS portion of this plasmid was determined and the plasmid was designated pGS4788 (FIG. 21(b)). The complete sequence of the synthetic GALGAP promotor (pGGAP) is shown in FIG. 20.

Detailed Description Text - DETX:

The design of the synthetic linker for joining two .alpha.-globin chains allows the inclusion of PstI and SpeI sites flanking a 30 bp sequence that includes the junction of the two .alpha.-globin coding sequences. Because we anticipate testing several different linker sequences, these sites will allow directional cloning of relatively short synthetic oligonucleotides encoding different linker sequences. Removal of the PstI and SpeI sites from the vector sequence is, therefore, necessary so that the sites in the coding region are usable. One .mu.g of the plasmid pGS4888 was digested with PstI and ethanol precipitated. The dry pellet was resuspended in 50 .mu.l of 33 mM Tris-acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT and 50 .mu.M of each dNTP (T4 polymerase buffer). Two units of T4 DNA polymerase were added and the reaction mixture incubated for 15 min at 37.degree. C. Na.sub.3 EDTA was added to 12.5 mM and the reaction mixture heated to 65.degree. C. for 15 min, phenol extracted and ethanol precipitated. The dry pellet was dissolved in 14 .mu.l of T4 DNA ligase buffer (BRL) and 1 .mu.l (10 units) of DNA ligase added. The ligation mixture was incubated at 4.degree. C. for 16 hr. A portion of the ligation reaction was used to transform E. coli DH5.alpha. and transformants were selected on LB-ampicillin plates. Plasmid DNA was prepared from 12 transformants. The DNA was analyzed by agarose gel electrophoresis of PstI digests. Five transformants had lost the PstI site and one of these was designated pGS1889. The SpeI site of this plasmid was removed as described above after digestion of pGS1889 with SpeI. A plasmid was identified that had lost both the PstI and the SpeI site and was designated pGS1989.

Detailed Description Text - DETX:

pSGE224 containing the 5 glycine di-beta linker sequences Parental plasmid is pSGE224 (42) The linker sequences are synthetic DNA.

US-PAT-NO: 5807689

DOCUMENT-IDENTIFIER: US 5807689 A

TITLE: Methods for identifying compounds that modulate metabotropic glutamate receptor activity

DATE-ISSUED: September 15, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-------------|-------|----------|---------|
| Daggett; Lorrie | San Diego | CA | N/A | N/A |
| Ellis; Steven B. | San Diego | CA | N/A | N/A |
| Liaw; Chen | San Diego | CA | N/A | N/A |
| Pontsler; Aaron | West Jordan | UT | N/A | N/A |
| Johnson; Edwin C. | San Diego | CA | N/A | N/A |
| Hess; Stephen D. | San Diego | CA | N/A | N/A |

APPL-NO: 08/ 486270

DATE FILED: June 6, 1995

PARENT-CASE:

RELATED APPLICATIONS This application is a divisional application of U.S. Ser. No. 08/367,264, filed Jan. 9, 1995, which is a 371 of International application PCT/US94/06273, filed Jun. 3, 1994, which is in turn a continuation-in-part application of U.S. Ser. No. 08/072,574, filed Jun. 4, 1993, now U.S. Pat. No. 5,521,297, the entire contents of which are hereby incorporated by reference.

US-CL-CURRENT: 435/7.8; 435/325 ; 435/69.1 ; 435/7.1 ; 435/7.2 ; 435/7.21

ABSTRACT:

In accordance with the present invention, there are provided nucleic acids encoding human metabotropic glutamate receptor subtypes and the proteins encoded thereby. In a particular embodiment, the invention nucleic acids encode mGluR1, mGluR2, mGluR3 and mGluR5 subtypes of human metabotropic glutamate receptors. In addition to being useful for the production of metabotropic glutamate receptor subtypes, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate related human receptor subunits. In addition to disclosing novel metabotropic glutamate receptor subtypes, the present invention also comprises methods for using such receptor subtypes to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function.

40 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Detailed Description Text - DETX:

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation.

Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the metabotropic glutamate receptor subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for potentially enhanced expression of metabotropic glutamate receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with Xenopus .beta.-globin gene 5' and 3' untranslated sequences, respectively. For example, metabotropic glutamate receptor subunit coding sequences can be incorporated into vector pSP64T (see Krieg and Melton (1984) in Nucleic Acids Research 12:7057-7070), a modified form of pSP64 (available from Promega, Madison, Wis.). The coding sequence is inserted between the 5' end of the P-globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. In vitro transcripts can then be generated from the resulting vector. The desirability of (or need for) such modifications may be empirically determined.

Detailed Description Text - DETX:

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human metabotropic glutamate receptor subtypes enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or in vitro

assay systems to determine the effects thereof.

US-PAT-NO: 5801019

DOCUMENT-IDENTIFIER: US 5801019 A

TITLE: DNA encoding fused alpha-beta globin pseudodimer and production of pseudotetrameric hemoglobin

DATE-ISSUED: September 1, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|------------|-------|----------|---------|
| Anderson; David C. | Lafayette | CO | N/A | N/A |
| Mathews; Antony James | Louisville | CO | N/A | N/A |

APPL-NO: 08/ 444939

DATE FILED: May 19, 1995

PARENT-CASE:

This is a division of application Ser. No. 07/789,179 filed Nov. 8, 1991, now U.S. Pat. No. 5,545,727, which is a CIP of Ser. No. 07/671,707 filed Apr. 1, 1991, now abandoned, which is a CIP of PCT/US90/02654 filed May 10, 1990, now abandoned, which is a CIP of (a) Ser. No. 07/374,161 filed Jun. 30, 1989, now abandoned, (b) Ser. No. 07/379,116 filed Jul. 13, 1989, now abandoned, and (c) Ser. No. 07/349,623 filed May 10, 1989, now abandoned, all hereby incorporated by reference herein.

US-CL-CURRENT: 435/69.6; 435/69.1 ; 435/69.7 ; 530/385 ; 536/23.4

ABSTRACT:

The alpha subunits of hemoglobin, which in nature are formed as separate polypeptide chains which bind noncovalently to the beta subunits, are here provided in the form of the novel molecule di-alpha globin, a single polypeptide chain defined by connecting the two alpha subunits either directly via peptide bond or indirectly by a flexible amino acid or peptide linker. Di-alpha globin may be combined in vivo or in vitro with beta globin and heme to form hemoglobin. Di-alpha globin is expressed by recombinant DNA techniques. Di-beta globin may be similarly obtained.

15 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 70

----- KWIC -----

Brief Summary Text - BSTX:

Saito, et al., J. Biochem., 101: 1281-88 (1987) expressed a **synthetic somatomedin C gene** in E. coli using a two cistron system. They theorized that the instability of somatomedin C, a basic polypeptide, might be overcome by complexing it with an acidic polypeptide. Thus, they constructed a two-cistron system which could express both polypeptides. The termination codon of the first cistron overlapped the initiation codon of the second cistron. The transformants accumulated Somatomedin C at high levels. However, the somatomedin C was recovered in the form of insoluble pellets (see page 1282).

Brief Summary Text - BSTX:

In one embodiment, the alpha- and beta-globin-like polypeptides are co-expressed in bacterial cells. The corresponding **genes may be included in the same synthetic** operon (i.e., driven by one promoter), or placed in separate operons with separate promoters (which may be the same or different). Preferably, expression of the alpha- and beta-globin is enhanced by placing a "ribosomal loader" sequence as hereafter described before each globin gene. This is particularly advantageous in the case of alpha globin which is more difficult to produce in quantity.

Drawing Description Text - DRTX:

FIG. 4: oligonucleotides for construction of **synthetic FX-alpha and FX-beta globin genes** (4a to 4c). The top strand is shown 5' to 3' and the bottom strand as 3' to 5'. Areas of overlap between complementary synthetic oligonucleotides are shown as areas where both strands are shown in the same case letters. The PstI site that joins FX-alpha and FX-beta occurs at the overlap of SJH I-35a and SJH I-36b.

Drawing Description Text - DRTX:

FIG. 5: **Synthetic gene** for expression of Met-FX-alpha and Met-FX-beta globin (5a to 5c). Region A contains the alpha globin gene and region B the beta globin gene. The location of the Factor X sequence and the two Shine-Delgarno sequences (SD#1 and SD#2) in both regions is indicated. Selected restriction sites are also found. The translated amino acid sequences for the ribosomal loader and Met-FX-alpha/and beta-globin are given.

Drawing Description Text - DRTX:

FIG. 12 Shows the **sequence of a preferred synthetic gene** for expression of (des-Val)-alpha-(GlyGly)-alpha and des-Val beta globin (12a to 12c). A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker.

The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual alpha (or di-alpha) and beta (or di-beta) globin chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially **synthetic gene** is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). For codon preferences in yeast, see the next section. Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the **promoter** associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the **codon preference** pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong **promoter** constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

The **synthetic FX-beta gene** sequence (included in FIG. 5) was constructed as follows: 100 pmole of the following oligo nucleotides were kinased in 3 separate reactions. Reaction 1 contained oligonucleotides SJH I-36b, c, d, e, and f. Reaction 2 contained SJH I-37a, b, c, and e. Reaction 3 contained SJH I-37d, f, and SJH I-38a. After combining the appropriate oligonucleotides, the solutions were lyophilized to dryness and resuspended in 16 uL of H.sub.2O. Two uL of 10.times. kinase buffer (0.5M Tris-HCl, pH7.4, 0.1M MgCl.sub.2), 0.5 uL of 100 mM DTT, and 1 uL of 1.0 mM ATP were then added. The reaction was initiated by addition of 1 uL (2U) of T4 polynucleotide kinase (IBI, Inc., New Haven, Conn.). After incubation at 37.degree. C. for 1 hour, the reactions were heated to 95.degree. C. for 10 minutes to inactivate the kinase. The

three reactions were combined and 100 pmoles of oligonucleotides SJH I-36a and SJH I-38b were added. After addition of 10 uL of 100 mM Tris, pH 7.8, 100 mM MgCl₂, the oligonucleotides were allowed to anneal by incubating at 65.degree. C. for 30 min, 37.degree. C. for 30 min, and 15.degree. C. for 1 hour. Annealed oligonucleotides were ligated by addition of ATP (1 mM, final) and DTT (10 mM final) and 4 uL (20U) T4 DNA ligase (IBI, Inc., New Haven, Conn.) and incubation at 15.degree. C. for 1 hour. Aliquots of this ligation mixture were then cloned directly into M13mp19 (see below).

Detailed Description Text - DETX:

Other hemoglobin mutants: The synthetic genes encoding Hemoglobin Cheverly (beta.sup.45 phe.fwdarw.ser) Hemoglobin Providence/MSR (beta.sup.82 lys.fwdarw.asp) and Hemoglobin beta.sup.67 val.fwdarw.ile and Hemoglobin Kansas (beta.sup.102 asn.fwdarw.thr) were prepared similarly except with synthetic oligonucleotides spanning the SacII.fwdarw.BglII, SalI.fwdarw.SpeI, NcoI.fwdarw.KpnI and SacI.fwdarw.SpeI restriction sites respectively (FIG. 7). Synthesis of the mutant oligonucleotides, restriction enzyme digestion, gel purification, and ligation conditions were identical to those used for Hemoglobin Beth Israel. All mutations were first cloned into plasmid pDL II-10a, appropriate clones were sequenced, and the mutated beta globin gene was subcloned into PstI and HindIII digested pDL II-66a. Plasmid sequencing was accomplished as described previously. E. coli cells were transformed, cultured, and induced as previously described. FX-hemoglobin mutants were purified by the method of Example 3. Oxygen binding of purified hemoglobin mutants is shown in Table 9.

Detailed Description Text - DETX:

The recognition site (FX)-encoding sequence could now be removed from pGEM FX-alpha and pGEM FX-beta to obtain pDL II-91f and pDL II-95a, respectively. The des-val alpha globin gene of pDL II-91f was recloned into pKK 223-3 to generate pDL III-1a, the gene being operably linked to the Tac promoter of pKK-223-3. The des-val beta globin gene of pDL II-95a was purified and inserted downstream of the des-val alpha globin gene of pDL III-1a to form a single transcriptional unit which would encode a polycistronic alpha globin/beta globin mRNA, see pDL III-14c. Finally, a synthetic oligonucleotide comprising the desired di-alpha linker encoding sequence and another copy of the alpha globin gene was inserted into pDL III-14c to create pDL III-47a, wherein a Tac promoter controls transcription of a di-alpha globin gene and a des-val beta globin gene.

Detailed Description Text - DETX:

The EagI and PstI restriction fragment containing most of the alpha globin gene from the plasmid pDL II-91f was gel purified and ligated to a synthetic DNA linker containing the sequence from the BstBI site of the alpha globin gene to the codon (wild-type Arginine) for its carboxyl terminus, a variable glycine-encoding linker (for example, FIG. 12, RGGV, a di-glycine followed by .alpha. Val; other possibilities include RGM, RGV, RGGV, etc., See Table 200),

and the codons for the amino terminal region of alpha globin to the EagI site (FIG. 12). After digesting this ligation mixture with Pst I, the resulting fragment was cloned into BstBI/PstI-cut pDL III-14C to create plasmid pDL III-47a (RGM-di-alpha). Plasmids pDL III-82a (RGGV-di-alpha), pDL IV-8a (RGV-di-alpha), pDL IV-976 (RV-di-alpha) and pDL IV-66a (RGGGV-di-alpha) are similarly constructed to incorporate the indicated changes in the di-alpha coding sequences.

Detailed Description Text - DETX:

Prior to inserting the globin genes into the vector it was necessary to incorporate the synthetic translational coupler sequence into the HpaI site of pPL-lambda-E. This was done by digestion of pPL-lambda-E with HpaI followed by blunt-end ligation of the co-translational coupler into the HpaI site of the vector. Ligation of the coupler to the blunt end resulted in destruction of the HpaI site. The ligation mixture was treated with HpaI to digest any plasmid remaining containing the HpaI site. E. coli N99Ci+ cells were transformed with the resulting reaction mixture. Clones were screened with EcoRI and Hind III restriction digests to identify clones containing the co-translational coupler in the proper orientation. DNA fragments of 522 bp and 4762 bp were observed for plasmid containing the desired orientation. To confirm the orientation of the coupler, the resulting plasmid was sequenced using a primer (5'CAATGGAAAGCAGCAAATCC-3') complementary to the sequence 30 base pairs upstream from the translational coupler sequence. The desired plasmid was denoted as pPL-lambda-E+TC.

Detailed Description Text - DETX:

This synthetic promoter consists of two functional parts, a regulatory sequence and sequence that allows efficient initiation of mRNA synthesis. One of the regulatory regions we chose includes the nucleotide sequence that confers positive regulation of transcription in the presence of galactose (M. Johnston and R. Davis, 1984. Molecular and Cellular Biology 4:1440-1448; L. Guarente et al., 1982, Proc Nat Acad Sci (U.S.A.) 79:7410-7414.). The transcriptional initiation site is derived from the consensus sequence for the S. cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene (GAP491) (L. McAlister and M. J. Holland, J. Biol Chem 260:15019-15027, 1983; J. P. Holland et al., J. Biol Chem 258:5291-5299, 1983).

Detailed Description Text - DETX:

ASSEMBLY OF THE SYNTHETIC GALACTOSE UPSTREAM4 ACTIVATOR (GAL.sub.UAS) SEQUENCE

Detailed Description Text - DETX:

The next step in the assembly of this hybrid promoter was to clone the SphI-Sall fragment containing the GAL.sub.UAS into pGS2888. pGS2888 was digested with SphI and Sall, phenolchloroform extracted and ethanol

precipitated. Fifty nanograms of SphI, Sall digested pGS2888 was incubated with 25 ng of the annealed, ligated GAL.sub.UAS mixture in 0.005 ml 1.times. ligase buffer containing 10 units of T4 DNA ligase. The ligation mixture was incubated overnight at 40.degree. C. and a portion used to transform E. coli DH5.alpha.. Ampicillin resistant clones were isolated and plasmid DNA prepared. The plasmid DNA (digested with XbaI and SphI) was analyzed by agarose gel electrophoresis. A plasmid containing a fragment of the expected size (.sup..about. 500 bp) was identified. The sequence of the putative GAL.sub.UAS portion of this plasmid was determined and the plasmid was designated pGS4788 (FIG. 21(b)). The complete sequence of the synthetic GALGAP promotor (pGGAP) is shown in FIG. 20.

Detailed Description Text - DETX:

The design of the synthetic linker for joining two .alpha.-globin chains allows the inclusion of PstI and SpeI sites flanking a 30 bp sequence that includes the junction of the two .alpha.-globin coding sequences. Because we anticipate testing several different linker sequences, these sites-will allow Directional cloning of relatively short synthetic oligonucleotides encoding different linker sequences. Removal of the PstI and SpeI sites from the vector sequence is, therefore, necessary so that the sites in the coding region are usable. One Mg of the plasmid pGS4888 was digested with PstI and ethanol precipitated. The dry pellet was resuspended in 50 .mu.l of 33 mM tris-acetate, pH7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT and 50 .mu.M of each dNTP (T4 polymerase buffer). Two units of T4 DNA polymerase were added and the reaction mixture incubated for 15 min at 37.degree. C. Na.sub.3 EDTA was added to 12.5 mM and the reaction mixture heated to 65.degree. C. for 15 min, phenol extracted and ethanol precipitated. The dry pellet was dissolved in 14 .mu.l of T4 DNA ligase buffer (BRL) and 1 .mu.l (10 units) of DNA ligase added. The ligation mixture was incubated a 4.degree. C. for 16 hr. A portion of the ligation reaction was used to transform E. coli DH5.alpha. and transformants were selected on LB-ampicillin plates. Plasmid DNA was prepared from 12 transformants. The DNA was analyzed by agarose gel electrophoresis of PstI digests. Five transformants had lost the PstI site and one of these was designated pGS1889. The SpeI site of this pLasmid was removed as described above after digestion of pGS1889 with SpeI. A plasmid was identified that had lost both the PstI and the SpeI site and was designated pGS1989.

Detailed Description Text - DETX:

pSGE224 containing the 5 glycine di-beta linker sequences Parental plasmid is pSGE224 (42) The linker sequences are synthetic DNA.

US-PAT-NO: 5798227

DOCUMENT-IDENTIFIER: US 5798227 A

TITLE: Co-expression of alpha and beta globins

DATE-ISSUED: August 25, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-----------|-------|----------|---------|
| Hoffman; Stephen J. | Denver | CO | N/A | N/A |
| Looker; Douglas L. | Lafayette | CO | N/A | N/A |
| Stetler; Gary L. | Denver | CO | N/A | N/A |
| Wagenbach; Michael | Osaka | N/A | N/A | JP |

APPL-NO: 08/ 446105

DATE FILED: May 19, 1995

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This is a division of application Ser. No. 07/789,179 filed Nov. 8, 1991, now U.S. Pat. No. 5,545,717, which is a CIP of 07/671,707 filed Apr. 1, 1991, now abandoned, which is a CIP of PCT/US90/02654 filed May 10, 1990, now abandoned, which is a CIP of (a) Ser. No. 07/374,161 filed Jun. 30, 1989, now abandoned, (b) Ser. No. 07/379,116 filed Jul. 13, 1989, now abandoned, and (c) Ser. No. 07/349,623 filed May 10, 1989, now abandoned, all hereby incorporated by reference herein. Hoffman and Nagai, U.S. Ser. No. 07/194,338, filed May 10, 1988, now U.S. Pat. No. 5,028,588, presently owned by Somatogen, Inc., relates to the use of low oxygen affinity and other mutant hemoglobins as blood substitutes, and to the expression of alpha and beta globin in nonerythroid cells. Hoffman and Nagai, U.S. Ser. No. 07/443,950, filed Dec. 1, 1989, discloses certain additional dicysteine hemoglobin mutants; it is a continuation-in-part of Ser. No. 07/194,338. Anderson, et al., HEMOGLOBINS AS DRUG DELIVERY AGENTS Atty. Docket.: ANDERSON5-USA, filed Nov. 8, 1991, discloses use of conjugation of hemoglobins with drugs as a means for delivery of the drug to a patient.

US-CL-CURRENT: 435/69.6; 435/252.33 ; 435/254.21 ; 435/320.1 ; 435/71.1 ; 435/71.2 ; 530/385

ABSTRACT:

The alpha subunits of hemoglobin, which in nature are formed as separate polypeptide chains which bind noncovalently to the beta subunits, are here provided in the form of the novel molecule di-alpha globin, a single polypeptide chain defined by connecting the two alpha subunits either directly via peptide bond or indirectly by a flexible amino acid or peptide linker. Di-alpha globin may be combined in vivo or in vitro with beta globin and heme to form hemoglobin. Di-alpha globin is expressed by recombinant DNA

techniques. Di-beta globin may be similarly obtained.

We further describe the production of tetrameric human hemoglobin and di-alpha/beta.sub.2 hemoglobin in the yeast *Saccharomyces cerevisiae*. The synthesis of the protein is directed by a **synthetic promotor consisting of two functional parts, an upstream activator sequence** (UAS) that confers inducible transcription by galactose from a consensus yeast transcriptional initiation site. The expression construct is designed such that translation is expected to initiate at the same position as the human wild-type genes for .alpha.- and .beta.-globin. Three different types of expression vectors have been used: (1) .alpha.-globin and .beta.-globin contained on two separate plasmids (pGS4688 and pGS4988) in a diploid yeast strain; (2) .alpha.-globin and .beta.-globin each contained on a single plasmid (pGS289 and pGS389) and expressed in either haploid or diploid strains; and (3) di-alpha-globin and beta globin contained on a single plasmid (pGS 3089)and expressed in haploid strains.

Finally, we describe the co-expression of alpha and beta globin chains. The chains are folded together and combined intracellularly with heme to form active tetrameric hemoglobin. The hemoglobin may be recovered from the cells' soluble fraction. The invention thus obviates the need to express alpha and beta globin separately, solubilize, renature and purify them, and combine them in vitro with heme to obtain an artificial hemoglobin. By way of comparison, the separately expressed beta globin known in the art is deposited in inclusion bodies. Polycistronic co-expression of alpha and beta globins is particularly preferred.

15 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 70

----- KWIC -----

Abstract Text - ABTX:

We further describe the production of tetrameric human hemoglobin and di-alpha/beta.sub.2 hemoglobin in the yeast *Saccharomyces cerevisiae*. The synthesis of the protein is directed by a **synthetic promotor consisting of two functional parts, an upstream activator sequence** (UAS) that confers inducible transcription by galactose from a consensus yeast transcriptional initiation site. The expression construct is designed such that translation is expected to initiate at the same position as the human wild-type genes for .alpha.- and .beta.-globin. Three different types of expression vectors have been used: (1) .alpha.-globin and .beta.-globin contained on two separate plasmids (pGS4688 and pGS4988) in a diploid yeast strain; (2) .alpha.-globin and .beta.-globin each contained on a single plasmid (pGS289 and pGS389) and expressed in either haploid or diploid strains; and (3) di-alpha-globin and beta globin contained on a single plasmid (pGS 3089)and expressed in haploid strains.

Brief Summary Text - BSTX:

Saito, et al., J. Biochem., 101: 1281-88 (1987) expressed a **synthetic somatomedin C gene** in E. coli using a two cistron system. They theorized that the instability of somatomedin C, a basic polypeptide, might be overcome by complexing it with an acidic polypeptide. Thus, they constructed a two-cistron system which could express both polypeptides. The termination codon of the first cistron overlapped the initiation codon of the second cistron. The transformants accumulated Somatomedin C at high levels. However, the somatomedin C was recovered in the form of insoluble pellets (see page 1282).

Brief Summary Text - BSTX:

In one embodiment, the alpha- and beta-globin-like polypeptides are co-expressed in bacterial cells. The corresponding **genes may be included in the same synthetic** operon (i.e., driven by one promoter), or placed in separate operons with separate promoters (which may be the same or different). Preferably, expression of the alpha- and beta-globin is enhanced by placing a "ribosomal loader" sequence as hereafter described before each globin gene. This is particularly advantageous in the case of alpha globin which is more difficult to produce in quantity.

Drawing Description Text - DRTX:

FIGS. 4a-4c: Oligonucleotides for construction of **synthetic FX-alpha and FX-beta globin genes**. The top strand is shown 5' to 3' and the bottom strand as 3' to 5'. Areas of overlap between complementary synthetic oligonucleotides are shown as areas where both strands are shown in the same case letters. The PstI site that joins FX-alpha and FX-beta occurs at the overlap of SJH I-35a and SJH I-36b.

Drawing Description Text - DRTX:

FIGS. 5a-5c: **Synthetic gene** for expression of Met-FX-alpha and Met-FX-beta globin (5a to 5c). Region A contains the alpha globin gene and region B the beta globin gene. The location of the Factor X sequence and the two Shine-Delgarno sequences (SD#1 and SD#2) in both regions is indicated. Selected restriction sites are also found. The translated amino acid sequences for the ribosomal loader and Met-FX-alpha/and beta-globin are given.

Drawing Description Text - DRTX:

FIGS. 12a-12c Shows the **sequence of a preferred synthetic gene** for expression of (des-Val)-alpha-(GlyGly)-alpha and des-Val beta globin (12a to 12c). A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains

an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual alpha (or di-alpha) and beta (or di-beta) globin chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially **synthetic gene** is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). For codon preferences in yeast, see the next section. Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the **promoter** associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the **codon preference** pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong **promoter** constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

The **synthetic FX-beta gene** sequence (included in FIG. 5) was constructed as follows: 100 pmole of the following oligo nucleotides were kinased in 3 separate reactions. Reaction 1 contained oligonucleotides SJH I-36b, c, d, e, and f. Reaction 2 contained SJH I-37a, b, c, and e. Reaction 3 contained SJH I-37d, f, and SJH I-38a. After combining the appropriate oligonucleotides, the solutions were lyophilized to dryness and resuspended in 16 uL of H.sub.2O. Two uL of 10.times. kinase buffer (0.5M Tris-HCl, pH 7.4, 0.1M MgCl.sub.2), 0.5 uL of 100 mM DTT, and 1 uL of 1.0 mM ATP were then added. The reaction was initiated by addition of 1 uL (2U) of T4 polynucleotide kinase (IBI, Inc., New Haven, Conn.). After incubation at 37.degree. C. for 1 hour, the reactions were heated to 95.degree. C. for 10 minutes to inactivate the kinase. The three reactions were combined and 100 pmoles of oligonucleotides SJH I-36a and

SJH I-38b were added. After addition of 10 uL of 100 mM Tris, pH 7.8, 100 mM MgCl₂, the oligonucleotides were allowed to anneal by incubating at 65.degree. C. for 30 min, 37.degree. C. for 30 min, and 15.degree. C. for 1 hour. Annealed oligonucleotides were ligated by addition of ATP (1 mM, final) and DTT (10 mM final) and 4 uL (20U) T4 DNA ligase (IBI, Inc., New Haven, Conn.) and incubation at 15.degree. C. for 1 hour. Aliquots of this ligation mixture were then cloned directly into M13mp19 (see below).

Detailed Description Text - DETX:

Other hemoglobin mutants: The **synthetic genes** encoding Hemoglobin Cheverly (beta.sup.45 phe.fwdarw.ser) Hemoglobin Providence/MSR (beta.sup.82 lys.fwdarw.asp) and Hemoglobin beta.sup.67 val.fwdarw.ile and Hemoglobin Kansas (beta.sup.102 asn.fwdarw.thr) were prepared similarly except with synthetic oligonucleotides spanning the SacII.fwdarw.BglII, Sall.fwdarw.SpeI, NcoI.fwdarw.KpnI and SacI.fwdarw.SpeI restriction sites respectively (FIG. 7). Synthesis of the mutant oligonucleotides, restriction enzyme digestion, gel purification, and ligation conditions were identical to those used for Hemoglobin Beth Israel. All mutations were first cloned into plasmid pDL II-10a, appropriate clones were sequenced, and the mutated beta globin gene was subcloned into PstI and HindIII digested pDL II-66a. Plasmid sequencing was accomplished as described previously. E. coli cells were transformed, cultured, and induced as previously described. FX-hemoglobin mutants were purified by the method of Example 3. Oxygen binding of purified hemoglobin mutants is shown in Table 9.

Detailed Description Text - DETX:

The recognition site (FX)-encoding sequence could now be removed from pGEM FX-alpha and pGEM FX-beta to obtain pDL II-91f and pDL II-95a, respectively. The des-val alpha globin gene of pDL II-91f was recloned into pKK 223-3 to generate pDL III-1a, the gene being operably linked to the Tac promoter of pKK-223-3. The des-val beta globin gene of pDL II-95a was purified and inserted downstream of the des-val alpha globin gene of pDL III-1a to form a single transcriptional unit which would encode a polycistronic alpha globin/beta globin mRNA, see pDL III-14c. Finally, a **synthetic oligonucleotide comprising the desired di-alpha linker encoding sequence** and another copy of the alpha globin gene was inserted into pDL III-14c to create pDL III-47a, wherein a Tac promoter controls transcription of a di-alpha globin gene and a des-val beta globin gene.

Detailed Description Text - DETX:

The EagI and PstI restriction fragment containing most of the alpha globin **gene from the plasmid pDL II-91f was gel purified and ligated to a synthetic DNA linker containing the sequence** from the BstBI site of the alpha globin gene to the codon (wild-type Arginine) for its carboxyl terminus, a variable glycine-encoding linker (for example, FIG. 12, RGGV, a di-glycine followed by a Val; other possibilities include RGM, RGV, RGGV, etc., See Table 200), and the codons for the amino terminal region of alpha globin to the EagI site (FIG.

12). After digesting this ligation mixture with Pst I, the resulting fragment was cloned into BstBI/PstI-cut pDL III-14C to create plasmid pDL III-47a (RGM-di-alpha). Plasmids pDL III-82a (RGGV-di-alpha), pDL IV-8a (RGV-di-alpha), pDL IV-976 (RV-di-alpha) and pDL IV-66a (RGGGV-di-alpha) were similarly constructed to incorporate the indicated changes in the di-alpha coding sequences.

Detailed Description Text - DETX:

Prior to inserting the globin genes into the vector it was necessary to incorporate the synthetic translational coupler sequence into the HpaI site of pPL-lambda-E. This was done by digestion of pPL-lambda-E with HpaI followed by blunt-end ligation of the co-translational coupler into the HpaI site of the vector. Ligation of the coupler to the blunt end resulted in destruction of the HpaI site. The ligation mixture was treated with HpaI to digest any plasmid remaining containing the HpaI site. E. coli N99Ci+ cells were transformed with the resulting reaction mixture. Clones were screened with EcoRI and Hind III restriction digests to identify clones containing the co-translational coupler in the proper orientation. DNA fragments of 522 bp and 4762 bp were observed for plasmid containing the desired orientation. To confirm the orientation of the coupler, the resulting plasmid was sequenced using a primer (5'CAATGGAAAGCAGCAAATCC-3') complementary to the sequence 30 base pairs upstream from the translational coupler sequence. The desired plasmid was denoted as pPL-lambda-E+TC.

Detailed Description Text - DETX:

This synthetic promoter consists of two functional parts, a regulatory sequence and sequence that allows efficient initiation of mRNA synthesis. One of the regulatory regions we chose includes the nucleotide sequence that confers positive regulation of transcription in the presence of galactose (M. Johnston and R. Davis, 1984. Molecular and Cellular Biology 4:1440-1448; L. Guarente et al., 1982, Proc Nat Acad Sci (USA) 79:7410-7414.). The transcriptional initiation site is derived from the consensus sequence for the S. cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene (GAP491) (L. McAlister and M. J. Holland, J. Biol Chem 260:15019-15027, 1983; J. P. Holland et al., J. Biol Chem 258:5291-5299, 1983).

Detailed Description Text - DETX:

Assembly of the Synthetic Galactose Upstream Activator (GAL.sub.UAS) Sequence

Detailed Description Text - DETX:

The next step in the assembly of this hybrid promoter was to clone the SphI-SalI fragment containing the GAL.sub.UAS into pGS2888. pGS2888 was digested with SphI and SalI, phenolchloroform extracted and ethanol precipitated. Fifty nanograms of SphI, SalI digested pGS2888 was incubated with 25 ng of the annealed, ligated GAL.sub.UAS mixture in 0.005 ml 1X ligase

buffer containing 10 units of T4 DNA ligase. The ligation mixture was incubated overnight at 4.degree. C. and a portion used to transform E. coli DH5.alpha.. Ampicillin resistant clones were isolated and plasmid DNA prepared. The plasmid DNA (digested with XbaI and SphI) was analyzed by agarose gel electrophoresis. A plasmid containing a fragment of the expected size (.about.500 bp) was identified. The sequence of the putative GAL.sub.UAS portion of this plasmid was determined and the plasmid was designated pGS4788 (FIG. 21(b)). The complete sequence of the synthetic GALGAP promotor (pGGAP) is shown in FIG. 20.

Detailed Description Text - DETX:

Removal of the PstI and SphI sites from pGS4888. The design of the synthetic linker for joining two .alpha.-globin chains allows the inclusion of PstI and SphI sites flanking a 30 bp sequence that includes the junction of the two .alpha.-globin coding sequences. Because we anticipate testing several different linker sequences, these sites will allow directional cloning of relatively short synthetic oligonucleotides encoding different linker sequences. Removal of the PstI and SphI sites from the vector sequence is, therefore, necessary so that the sites in the coding region are usable. One .mu.g of the plasmid pGS4888 was digested with PstI and ethanol precipitated. The dry pellet was resuspended in 50 .mu.l of 33 mM Tris-acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT and 50 .mu.M of each dNTP (T4 polymerase buffer). Two units of T4 DNA polymerase were added and the reaction mixture incubated for 15 min at 37.degree. C. Na.sub.3 EDTA was added to 12.5 mM and the reaction mixture heated to 65.degree. C. for 15 min, phenol extracted and ethanol precipitated. The dry pellet was dissolved in 14 .mu.l of T4 DNA ligase buffer (BRL) and 1 .mu.l (10 units) of DNA ligase added. The ligation mixture was incubated a 4.degree. C. for 16 hr. A portion of the ligation reaction was used to transform E. coli DH5.alpha. and transformants were selected on LB-ampicillin plates. Plasmid DNA was prepared from 12 transformants. The DNA was analyzed by agarose gel electrophoresis of PstI digests. Five transformants had lost the PstI site and one of these was designated pGS1889. The SphI site of this plasmid was removed as described above after digestion of pGS1889 with SphI. A plasmid was identified that had lost both the PstI and the SphI site and was designated pGS1989.

Detailed Description Paragraph Table - DETL:

| | |
|--------------------|--|
| AR, TS, ROP+, LAC- | 1. pDL II-62m pKK223-3 containing FX-A |
| AR, TS, ROP+, LAC- | 2. pDL II-10a pKK223-2 containing FX-B |
| AR, TS, ROP+, LAC- | 3. PDL II-66A Parental plasmids are (1) and (2); contains both FX-A and FX-B in single operon |
| AR, TS, ROP+, LAC- | 4. pGEM FX-A Parental plasmids are (1) and pGem1 which is commercially available from Promega Corporation, 2800 Woods Hollow Rd., Madison, WI 53711. |
| AR, TS, ROP+, LAC- | 5. pGEM FX-B pGEM containing FX-B, AR Parental plasmids are (2) and pGEM1 |
| AR, TS, ROP+, LAC- | 5a. PGEM di-alpha Parentals are PGEM1 (see discussion of #4) and (2). The SmaI/PstI fragment of (29), containing the di-alpha gene, is excised and ligated into SmaI/PstI-cut pGEM1. |
| AR, TS, ROP+, LAC- | 6. pDL II-83a Parental is 4; contains DFX-A |
| AR, TS, ROP+, LAC- | 7. pDL III-6f Parental is 5; contains DFX-B, AR |
| AR, TS, ROP+, LAC- | 8. PDL II-86c Parentals are pKK223-3 and (6), contains DFX-A, AR, TS, ROP+, LAC- |
| AR, TS, ROP+, LAC- | 9. pDL III-13e |

Parentals are (7) and (8), pKK223-3 containing both DFX-A and DFX-B, AR, TS, ROP+, LAC- 10. pDL II-91f Parental is (4) contains DV-A1 AR 11. pDL II-95a Parental is (7) contains DV-B, AR 12. pDL III-1a Parentals are pKK223-3 and (10), contains DV-A AR, TS, ROP+, LAC- 13. pDL III-14c Parentals are (11) and (12), contains DV-A and DV-B, AR, TS, ROP+, LAC- 13a. PDL III-38b Parentals are (11) and (23) AR, TS, ROP+, LAC- 14. pDL III-47a Parental is (13), contains RGM-di-alpha and DV- B, AR, TS, ROP+, LAC- 15. pDL III-82A Parental is (13), contains RGGV-di-alpha and DV- B AR, TS, ROP+, LAC- 16. pDL IV-8a Parental is (13), contains RGV-di-alpha and DV- B, AR, TS, ROP+, LAC- 17. pDL IV-47b Parental is (13), contains RV-di-alpha and DV-B AR, TS, ROP+, LAC- 18. pDL IV-66a Parental is (13), contains RGGGV-di-alpha and DV-B, AR, TS, ROP+, LAC- 19. pDL IV-3a Parental is (15), ROP gene is inactivated by insertion of a Not I linker into the PvuII site within the ROP gene AR, TS, ROP-, LAC- 20. pDL IV-38a Parental is (15), contains the Nagai mutation in DV-B, AR, TS, ROP+, LAC- 21. pDL IV-58f Parental is (20), ROP gene inactivated as in (19), AR, TS, ROP-, LAC- 22. pDL IV-59a Parental is (21) and pBR322, which is commercially available from a number of different suppliers. Contains a functional TR gene constructed in the following manner: The EcoRI site of pBR322 was changed to a BamHI linker. This permitted the removal of the 5' end of the TR gene from pBR322 as a BamHI fragment. This fragment was then inserted into the BamHI site located at the junction of the TAC promoter and the inactive TR gene of pKK223-3. Insertion of this fragment reactivates the TR gene if the fragment inserts in the proper orientation. Selection of colonies on tetracycline plates assures presence of the fragment in the proper orientation. AR, TR, ROP-, LAC- 23. pJR V-83a Parental is (11), contains DV-B with the Presbyterian mutation (asn108->lys), AR, TS, ROP+, LAC- 24. pJR VI-29a Parentals are (15) and (23), contains RGGV-di-alpha and DV-B with Presbyterian mutation, AR, TS, ROP+, LAC- 25. pJR VI-53b Parental is (24), made TR by insertion of BAMHI fragment, AR, TR, ROP+, LAC- 26. pJR VI-61a Parental is (25), made ROP- by insertion of Not I linker into PvuII site AR, TR, ROP-, LAC- 27. pDL V-4a Parentals are (16) and (26), contains RGV-di-alpha and DV-B with Presbyterian mutation AR, TS, ROP-, LAC- 28. pDL V-10a Parental is (27), insertion of BamHI fragment to convert to TR AR, TR, ROP-, LAC- 29. pDL V-16d (also named pSGE1.1E4 or simply pSGE1.1) Parental is (28), contains LACI gene inserted into Not I site. LACI gene obtained using following protocol: Polymerase chain reaction (PCR) primers containing NOTI sites at their 5' ends were used to amplify the LACI gen. Following gel purification, the gene was inserted into the NOT I site of pDL V-1a AR, TR, ROP-, LAC+ Several other plasmid constructs have been designed to facilitate the incorporation of a second beta globin gene under regulation of its own TAC promoter. 30. pDL IV-64a Parental is (14), contains beta globin under regulation of a synthetic TAC promoter AR, TS, ROP+, LAC- 31. PDL IV-67a Parental plasmids are (14) and (30), contains di-alpha under regulation of one pTAC, and DV-B under regulation of a second PTAC, DV-B is adjacent to di-alpha AR, TS, ROP-, LAC- 32. pJR VI-54a Parental plasmids are (14) and (30), contains di-alpha and DV-B under regulation of one pTAC and a second DV-B under regulation of another pTAC. The second DV-B is inserted into the Pvu II site of the plasmid AR, TS, ROP-, LAC- 33. pPL Lambda, Commercially available plasmid from Pharmacia LKB (see above); contains pL promoter and coding region for N protein of lambda which can be used for expression of fusion or translationally coupled recombinant proteins. 34. pPL-alpha/beta Parental plasmids are (13) and (33), contains DV-A and DV-B AR, ROP+ 35.

pPL-di-alpha/beta Parental is (34), contains RGV-di-alpha and DV-B AR, TS, ROP+, LAC- 36. pSGE0.1-L0 Parental plasmid is (35), ROP gene inactivated by insertion of NotI linker into PvuII site in ROP gene AR, ROP- 37. pSGE0.1E4 Parentals are pSGE1.1E4 (29) and pDL II-95a (11), contains di-alpha globin gene followed by wild type beta globin gene. The wild type beta globin gene from pDL II-95a was excised with PstI and HindIII, gel purified, and ligated into pSGE1.1E4 from which the beta Presbyterian gene had been removed with the same restriction enzymes. 38. PSGE0.0E4 Parentals are pSGE0.1E4 (37) and pDL II-91f (10), contains di-alpha globin gene followed by wild type beta globin gene. The alpha globin gene from pDL II-91f was excised with SmaI and PstI, gel purified, and ligated into pSGE0.1E4 from which the di-alpha globin gene removed using the same restriction enzymes. 39. pPL lambda + TC Parental is pPL lambda (33). pPL lambda was linearized with the restriction enzyme HpaI and synthetic oligonucleotides encoding the translational coupler region, and a portion of the 5' coding region of alpha globin ligated into the HpaI site. 40. PSGE1.0E4 Parentals are pSGE1.1E4 (29) and pSGE0.0E4 (38), contains a single alpha globin followed by beta globin gene containing the Presbyterian mutation. pSGE0.0E4 was digested with SmaI and PstI, the alpha globin gene gel purified, and ligated into pSGE1.1E4 from which the di-alpha gene had been removed with the same restriction enzymes. 41. pSGE223 Parental is commercially available pGEM-1. NheI restriction site has been eliminated by digestion with NheI, T4 polymerase filling, and ligation. 42. pSGE224 pSGE223 containing the beta Presbyterian gene. Parentals are pSGE223 (41) and pSGE1.1E4 (29) 43. pSGE229 pSGE224 containing the 5 glycine di-beta linker sequences parental plasmid is pSGE224 (42) The linker sequences are synthetic DNA. 44. pSGE234 contains the di-beta globin gene. Parentals are pSGE224 (42) and pSGE229 (43). The NheI fragment from pSGE229 contains the beta Presbyterian gene and the 5 glycine di-beta linker was ligated into NheI digested PSGE224. 45. pSge1.1E5 Parental is pSGE1.1E4 (29). 5' end of the tet R gene was removed by digestion with BamHI. 45a. pSGE0.0E5 Parental is pSGE0.0E4 (38). BamHI fragment encoding 5' end of tet R gene has been removed. 46. pSGE1.0E5 single alpha globin gene followed by beta Presbyterian gene. Parentals are pSGE0.0E5 (45a) and pJRV-83a (23). The beta globin gene containing the Presbyterian mutation was excised from (23) and ligated into (45a), from which the wild type beta globin gene had been removed with the same two restriction enzymes. 47. pSGE1.05E5 single alpha globin gene followed by 5 glycine linked di-beta gene. Parentals are pSGE234 (44) and pSGE1.0E5 (46). The PstI into HindIII fragment from pSGE234 containing the di-beta gene was ligated into pSGE1.0E5 from which the single beta globin gene had been removed using the same restriction enzymes. 48. pSGE1.05E4 Parentals are pSGE1.05E5 (47) and pSGE1.1E4 (29). The BamHI fragment encoding the 5' end of the tet R gene from pSGE1.1E4 was ligated into the BamHI site of pSGE1.05E5. YEAST VECTORS 49. pSK+

US-PAT-NO: 5773268

DOCUMENT-IDENTIFIER: US 5773268 A

TITLE: Chromosome 21 gene marker, compositions and methods using same

DATE-ISSUED: June 30, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-------------|-------|----------|---------|
| Korenberg; Julie R. | Los Angeles | CA | N/A | N/A |
| Yamakawa; Kazuhiro | Los Angeles | CA | N/A | N/A |

APPL-NO: 08/ 337690

DATE FILED: November 9, 1994

US-CL-CURRENT: 435/6; 435/252.3 ; 435/252.33 ; 435/320.1 ; 435/325 ; 435/348
; 435/349 ; 435/350 ; 435/352 ; 435/357 ; 435/366 ; 435/372.3 ; 514/44
; 536/23.1 ; 536/23.5

ABSTRACT:

The present invention provides isolated nucleic acids encoding human EHOC-1 protein and isolated receptor proteins encoded thereby. Further provided are vectors containing invention nucleic acids, probes that hybridize thereto, host cells transformed therewith, antisense oligonucleotides thereto and compositions containing, antibodies that specifically bind to invention polypeptides and compositions containing, as well as transgenic non-human mammals that express the invention protein.

19 Claims, 4 Drawing figures

Exemplary Claim Number: 1,19

Number of Drawing Sheets: 3

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Detailed Description Text - DETX:

Vectors that contain both a **promoter** and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate

alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NnAChR .alpha.9 subunit in order to enhance transcription (e.g., the codon preference of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Also provided are vectors comprising the invention nucleic acids. Examples of vectors are viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

US-PAT-NO: 5744329

DOCUMENT-IDENTIFIER: US 5744329 A

TITLE: DNA encoding fused di-beta globins and production of pseudotetrameric hemoglobin

DATE-ISSUED: April 28, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|------------|-------|----------|---------|
| Hoffman; Stephen J. | Denver | CO | N/A | N/A |
| Looker; Douglas L. | Lafayette | CO | N/A | N/A |
| Rosendahl; Mary S. | Broomfield | CO | N/A | N/A |
| Stetler; Gary L. | Denver | CO | N/A | N/A |
| Wagenbach; Michael | Osaka | N/A | N/A | JP |
| Anderson; David C. | Lafayette | CO | N/A | N/A |
| Mathews; Antony James | Louisville | CO | N/A | N/A |
| Nagai; Kiyoshi | Cambridge | N/A | N/A | GB2 |

APPL-NO: 08/ 444942

DATE FILED: May 19, 1995

PARENT-CASE:

This is a division of Ser. No. 07/789,179 filed Nov. 8, 1991, now U.S. Pat. No. 5,545,727, which is a CIP of Ser. No. 07/671,707 filed Apr. 1, 1991, now abandoned, which is a CIP of PCT/US90/02654 filed May 10, 1990, now abandoned, which is a CIP of (a) Ser. No. 07/374,161 filed Jun. 30, 1989, now abandoned, (b) Ser. No. 07/379,116 filed Jul. 13, 1989, now abandoned, and (c) Ser. No. 07/349,623 filed May 10, 1989, now abandoned, all hereby incorporated by reference herein.

US-CL-CURRENT: 435/69.6; 435/69.1 ; 435/69.7 ; 530/385 ; 536/23.4

ABSTRACT:

The alpha subunits of hemoglobin, which in nature are formed as separate polypeptide chains which bind noncovalently to the beta subunits, are here provided in the form of the novel molecule di-alpha globin, a single polypeptide chain defined by connecting the two alpha subunits either directly via peptide bond or indirectly by a flexible amino acid or peptide linker. Di-alpha globin may be combined in vivo or in vitro with beta globin and heme to form hemoglobin. Di-alpha globin is expressed by recombinant DNA techniques. Di-beta globin may be similarly obtained.

19 Claims, 72 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 70

----- KWIC -----

Brief Summary Text - BSTX:

Saito, et al., J. Biochem., 101: 1281-88 (1987) expressed a **synthetic somatomedin C gene** in E. coli using a two cistron system. They theorized that the instability of somatomedin C, a basic polypeptide, might be overcome by complexing it with an acidic polypeptide. Thus, they constructed a two-cistron system which could express both polypeptides. The termination codon of the first cistron overlapped the initiation codon of the second cistron. The transformants accumulated Somatomedin C at high levels. However, the somatomedin C was recovered in the form of insoluble pellets (see page 1282).

Brief Summary Text - BSTX:

In one embodiment, the alpha- and beta-globin-like polypeptides are co-expressed in bacterial cells. The corresponding **genes may be included in the same synthetic** operon (i.e., driven by one promoter), or placed in separate operons with separate promoters (which may be the same or different). Preferably, expression of the alpha- and beta-globin is enhanced by placing a "ribosomal loader" sequence as hereafter described before each globin gene. This is particularly advantageous in the case of alpha globin which is more difficult to produce in quantity.

Drawing Description Text - DRTX:

FIG. 4 Oligonucleotides for construction of **synthetic FX-alpha and FX-beta globin genes**. The top strand is shown 5' to 3' and the bottom strand as 3' to 5'. Areas of overlap between complementary synthetic oligonucleotides are shown as areas where both strands are shown in the same case letters. The PstI site that joins FX-alpha and FX-beta occurs at the overlap of SJH I-35a and SJH I-36b.

Drawing Description Text - DRTX:

FIG. 5 **Synthetic gene** for expression of Met-FX-alpha and Met-FX-beta globin (5a to 5c). Region A contains the alpha globin gene and region B the beta globin gene. The location of the Factor X sequence and the two Shine-Delgarno sequences (SD#1 and SD#2) in both regions is indicated. Selected restriction sites are also found. The translated amino acid sequences for the ribosomal loader and Met-FX-alpha/and beta-globin are given.

Drawing Description Text - DRTX:

FIG. 12 Shows the sequence of a preferred synthetic gene for expression of (des-Val)-alpha-(GlyGly)-alpha and des-Val beta globin (12a to 12c). A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. A and B are connected at the PstI site to form a single polycistronic operon.

Drawing Description Text - DRTX:

The DNA sequences encoding the individual alpha (or di-alpha) and beta (or di-beta) globin chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified. by excising the introns. Use of an at least partially synthetic gene is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). For codon preferences in yeast, see the next section. Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Drawing Description Text - DRTX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the promoter associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the codon preference pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong promoter constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

The synthetic FX-beta gene sequence (included in FIG. 5) was constructed as follows: 100 pmole of the following oligo nucleotides were kinased in 3 separate reactions. Reaction 1 contained oligonucleotides SJH I-36b, c, d, e, and f. Reaction 2 contained SJH I-37a, b, c, and e. Reaction 3 contained SJH I-37d, f, and SJH I-38a. After combining the appropriate oligonucleotides, the

solutions were lyophilized to dryness and resuspended in 16 μ L of H₂O. Two μ L of 10-times kinase buffer (0.5 M Tris-HCl, pH 7.4, 0.1 M MgCl₂), 0.5 μ L of 100 mM DTT, and 1 μ L of 1.0 mM ATP were then added. The reaction was initiated by addition of 1 μ L (2U) of T4 polynucleotide kinase (IBI, Inc., New Haven, Conn.). After incubation at 37°C for 1 hour, the reactions were heated to 95°C for 10 minutes to inactivate the kinase. The three reactions were combined and 100 pmoles of oligonucleotides SJH I-36a and SJH I-38b were added. After addition of 10 μ L of 100 mM Tris, pH 7.8, 100 mM MgCl₂, the oligonucleotides were allowed to anneal by incubating at 65°C for 30 min, 37°C for 30 min, and 15°C for 1 hour. Annealed oligonucleotides were ligated by addition of ATP (1 mM, final) and DTT (10 mM final) and 4 μ L (20 U) T4 DNA ligase (IBI, Inc., New Haven, Conn.) and incubation at 15°C for 1 hour. Aliquots of this ligation mixture were then cloned directly into M13mp19 (see below).

Detailed Description Text - DETX:

Other hemoglobin mutants: The **synthetic genes** encoding Hemoglobin Cheverly (beta.sup.45 phe.fwdarw.ser) Hemoglobin Providence/MSR (beta.sup.82 lys.fwdarw.asp) and Hemoglobin beta.sup.67 val.fwdarw.ile and Hemoglobin Kansas (beta.sup.102 asn.fwdarw.thr) were prepared similarly except with synthetic oligonucleotides spanning the SacII.fwdarw.BqIII, SalI.fwdarw.SpeI, NcoI.fwdarw.KpnI and SacI.fwdarw.SpeI restriction sites respectively (FIG. 7). Synthesis of the mutant oligonucleotides, restriction enzyme digestion, gel purification, and ligation conditions were identical to those used for Hemoglobin Beth Israel. All mutations were first cloned into plasmid pDL II-10a, appropriate clones were sequenced, and the mutated beta globin gene was subcloned into PstI and HindIII digested pDL II-66a. Plasmid sequencing was accomplished as described previously. E. coli cells were transformed, cultured, and induced as previously described. FX-hemoglobin mutants were purified by the method of Example 3. Oxygen binding of purified hemoglobin mutants is shown in Table 9.

Detailed Description Text - DETX:

The recognition site (FX)-encoding sequence could now be removed from pGEM FX-alpha and pGEM FX-beta to obtain pDL II-91f and pDL II-95a, respectively. The des-val alpha globin gene of pDL II-91f was recloned into pKK 223-3 to generate pDL III-1a, the gene being operably linked to the Tac promoter of pKK-223-3. The des-val beta globin gene of pDL II-95a was purified and inserted downstream of the des-val alpha globin gene of pDL III-1a to form a single transcriptional unit which would encode a polycistronic alpha globin/beta globin mRNA, see pDL III-14c. Finally, a **synthetic oligonucleotide comprising the desired di-alpha linker encoding sequence** and another copy of the alpha globin gene was inserted into pDL III-14c to create pDL III-47a, wherein a Tac promoter controls transcription of a di-alpha globin gene and a des-val beta globin gene.

Detailed Description Text - DETX:

The EagI and PstI restriction fragment containing most of the alpha globin **gene from the plasmid pDL II-91f was gel purified and ligated to a synthetic DNA linker containing the sequence** from the BstBI site of the alpha globin gene to the codon (wild-type Arginine) for its carboxyl terminus, a variable glycine-encoding linker (for example, FIG. 12, RGGV, a di-glycine followed by .alpha. Val; other possibilities include RGM, RGV, RGGV, etc., See Table 200), and the codons for the amino terminal region of alpha globin to the EagI site (FIG. 12). After digesting this ligation mixture with Pst I, the resulting fragment was cloned into BstBI/PstI-cut pDL III-14C to create plasmid pDL III-47a (RGM-di-alpha). Plasmids pDL III-82a (RGGV-di-alpha), pDL IV-8a (RGV-di-alpha), pDL IV-976 (RV-di-alpha) and pDL IV-66a (RGGGV-di-alpha) were similarly constructed to incorporate the indicated changes in the di-alpha coding sequences.

Detailed Description Text - DETX:

Prior to inserting the globin **genes into the vector it was necessary to incorporate the synthetic translational coupler sequence** into the HpaI site of pPL-lambda-E. This was done by digestion of pPL-lambda-E with HpaI followed by blunt-end ligation of the co-translational coupler into the HpaI site of the vector. Ligation of the coupler to the blunt end resulted in destruction of the HpaI site. The ligation mixture was treated with HpaI to digest any plasmid remaining containing the HpaI site. E. coli N99Ci+ cells were transformed with the resulting reaction mixture. Clones were screened with EcoRI and Hind III restriction digests to identify clones containing the co-translational coupler in the proper orientation. DNA fragments of 522 bp and 4762 bp were observed for plasmid containing the desired orientation. To confirm the orientation of the coupler, the resulting plasmid was sequenced using a primer (5'CAATGGAAAGCAGCAAATCC-3') complementary to the sequence 30 base pairs upstream from the translational coupler sequence. The desired plasmid was denoted as pPL-lambda-E+TC.

Detailed Description Text - DETX:

This **synthetic promoter consists of two functional parts, a regulatory sequence** and sequence that allows efficient initiation of mRNA synthesis. One of the regulatory regions we chose includes the nucleotide sequence that confers positive regulation of transcription in the presence of galactose (M. Johnston and R. Davis, 1984. Molecular and Cellular Biology 4:1440-1448; L. Guarente et al., 1982, Proc Nat Acad Sci (USA) 79:7410-7414.). The transcriptional initiation site is derived from the consensus sequence for the S.cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene (GAP491) (L. McAlister and M. J. Holland, J. Biol Chem 260:15019-15027, 1983; J. P. Holland et al., J. Biol Chem 258:5291-5299, 1983).

Detailed Description Text - DETX:

ASSEMBLY OF THE **SYNTHETIC GALACTOSE UPSTREAM ACTIVATOR (GAL.sub.UAS) SEQUENCE**

Detailed Description Text - DETX:

The next step in the assembly of this hybrid promoter was to clone the SphI - Sall fragment containing the GAL.sub.UAS into pGS2888. pGS2888 was digested with SphI and Sall, phenol-chloroform extracted and ethanol precipitated. Fifty nanograms of SphI, Sall digested pGS2888 was incubated with 25 ng of the annealed, ligated GAL.sub.UAS mixture in 0.005 ml 1X ligase buffer containing 10 units of T4 DNA ligase. The ligation mixture was incubated overnight at 4.degree. C. and a portion used to transform E. coli DH5.alpha.. Ampicillin resistant clones were isolated and plasmid DNA prepared. The plasmid DNA (digested with XbaI and SphI) was analyzed by agarose gel electrophoresis. A plasmid containing a fragment of the expected size (.sup.- 500bp) was identified. The sequence of the putative GAL.sub.UAS portion of this plasmid was determined and the plasmid was designated pGS4788 (FIG. 21(b)). The complete sequence of the synthetic GALGAP promotor (PGGAP) is shown in FIG. 20.

Detailed Description Text - DETX:

The design of the synthetic linker for joining two .alpha.-globin chains allows the inclusion of PstI and SpeI sites flanking a 30 bp sequence that includes the junction of the two .alpha.-globin coding sequences. Because we anticipate testing several different linker sequences, these sites will allow directional cloning of relatively short synthetic oligonucleotides encoding different linker sequences. Removal of the PstI and SpeI sites from the vector sequence is, therefore, necessary so that the sites in the coding region are usable. One .mu.g of the plasmid pGS4888 was digested with PstI and ethanol precipitated. The dry pellet was resuspended in 50 .mu.l of 33 mM Tris-acetate, pH7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT and 50 .mu.M of each dNTP (T4 polymerase buffer). Two units of T4 DNA polymerase were added and the reaction mixture incubated for 15 min at 37.degree. C. Na.sub.3 EDTA was added to 12.5 mM and the reaction mixture heated to 65.degree. C. for 15 min, phenol extracted and ethanol precipitated. The dry pellet was dissolved in 14 .mu.l of T4 DNA ligase buffer (BRL) and 1 .mu.l (10 units) of DNA ligase added. The ligation mixture was incubated at 4.degree. C. for 16 hr. A portion of the ligation reaction was used to transform E. coli DH5.alpha. and transformants were selected on LB-ampicillin plates. Plasmid DNA was prepared from 12 transformants. The DNA was analyzed by agarose gel electrophoresis of PstI digests. Five transformants had lost the PstI site and one of these was designated pGS1889. The SpeI site of this plasmid was removed as described above after digestion of pGS1889 with SpeI. A plasmid was identified that had lost both the PstI and the SpeI site and was designated pGS1989.

Detailed Description Text - DETX:

Parental plasmid is pSGE224 (42) The linker sequences are synthetic DNA.

US-PAT-NO: 5739011

DOCUMENT-IDENTIFIER: US 5739011 A

TITLE: DNA for the production of multimeric hemoglobins

DATE-ISSUED: April 14, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|------------|-------|----------|---------|
| Anderson; David C. | San Bruno | CA | N/A | N/A |
| Mathews; Antony James | Louisville | CO | N/A | N/A |
| Stetler; Gary L. | Boulder | CO | N/A | N/A |

APPL-NO: 08/ 443890

DATE FILED: May 31, 1995

PARENT-CASE:

This application is a continuation of Ser. No. 08/240,712, filed May 9, 1994, now U.S. Pat. No. 5,595,903, which is a continuation-in-part of Ser. No. 07/789,179, filed Nov. 8, 1991, now U.S. Pat. No. 5,543,727, which is a continuation-in-part of Ser. No. 07/671,707, filed Apr. 1, 1991, now abandoned, which is the national stage of PCT/US90/02654, filed May 10, 1990, which is a continuation-in-part of (a) Looker and Hoffman, U.S. Ser. No. 07/374,161, DI-ALPHA AND DI-BETA GLOBIN LIKE POLYPEPTIDES AND USES THEREFOR, filed Jun. 30, 1989 now abandoned; (b) Stetler and Wagenbach, U.S. Ser. No. 07/379,116, PRODUCTION OF HUMAN HEMOGLOBIN BY TRANSFORMED YEAST CELLS, filed Jul. 13, 1989 now abandoned; and (c) Hoffman, Looker, Rosendahl and Stetler, U.S. Ser. No. 07/349,623, POLYCISTRONIC CO-EXPRESSION OF THE ALPHA- AND BETA-GLOBINS AND IN VIVO ASSEMBLY OF BIOLOGICALLY ACTIVE, TETRAMERIC HEMOGLOBIN, filed May 10, 1989 now abandoned; all owned by Somatogen, Inc.

US-CL-CURRENT: 435/69.6; 435/254.11 ; 435/257.3 ; 435/320.1 ; 435/325 ; 530/385 ; 536/23.5

ABSTRACT:

DNA molecules which encode pseudodimeric globin-like polypeptides with an asymmetric cysteine mutation suitable for crosslinking two tetramers, or which encode pseudooligomeric globin-like polypeptides comprising four or more globin-like domains, are useful in the preparation of multimeric hemoglobin-like proteins.

37 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Drawing Description Text - DRTX:

FIGS. 2a-2e Show the **sequence [SEQ ID NO:1] of a preferred synthetic gene** for expression of (des-Val)-alpha-(Gly)-alpha and des-Val beta globin. This gene is carried by pSGE1.1E4. A show the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) (SEQ ID NO:25) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The residues are numbered 1 to 141 (SEQ ID NO:26). The second alpha globin sequence begins Val-Leu, immediately after the underlined "Gly-Gly" linker. The residues are numbered 1' to 141' (SEQ ID NO:27). Start and stop codons are underlined. B show the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin.

Detailed Description Text - DETX:

The DNA **sequences encoding the individual polypeptide chains may be of genomic, cDNA and synthetic** origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially **synthetic gene** is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second., the codon selection may be made to optimize expression in a selected host. For codon preferences in E. coli, see Konigsberg, et al., PNAS, 80:687-91 (1983). Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in S. cerevisiae is primarily affected by the strength of the **promoter** associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the **codon preference** pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong **promoter** constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

This is a derivative of pKK223-3 (Pharmacia LKB, Piscataway, N.J., U.S.A.) and pGEM1 (Promega Corp., Madison, Wis., U.S.A.) which carries synthetic genes for des-Val alpha globin and des-Val beta globin as part of a polycistronic operon driven by a single Tac promoter.

Other Reference Publication - OREF:

Schoner, B. et al/Expression of Eukaryotic Genes in Escherichia coli with a Synthetic Two Cistron System/Methods in Enzymology/vol. 153 Recombinant DNA Part D/Ed: Ray Wu & L. Grossman/Academic Press. Inc./NY/(1987), 401-416.

US-PAT-NO: 5683912

DOCUMENT-IDENTIFIER: US 5683912 A

TITLE: Cloning and expression of a novel acetylcholine-gated ion channel receptor subunit

DATE-ISSUED: November 4, 1997

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------------|-----------|-------|----------|---------|
| Elgoyhen; Ana Belen | Del Mar | CA | N/A | N/A |
| Johnson; David S. | La Jolla | CA | N/A | N/A |
| Boulter; James Richard | San Diego | CA | N/A | N/A |
| Heinemann; Stephen Fox | La Jolla | CA | N/A | N/A |

APPL-NO: 08/ 278635

DATE FILED: July 21, 1994

PARENT-CASE:

RELATED INVENTIONS This invention is related to U.S. Ser. No. 07/898,185, filed Jun. 12, 1992, now U.S. Pat. No. 5,371,188, which is a continuation of U.S. Ser. No. 07/664,473, filed Mar. 4, 1991, now abandoned, which is, in turn, a continuation of U.S. Ser. No. 07/321,384, filed Mar. 14, 1989, now abandoned, which is, in turn, a continuation-in-part of U.S. Ser. No. 07/170,295, filed Mar. 18, 1988, now abandoned, all of which are hereby incorporated by reference herein in their entirety.

US-CL-CURRENT: 435/252.3; 435/320.1 ; 435/69.1 ; 536/23.5

ABSTRACT:

The present invention provides isolated nucleic acids encoding alpha9 nicotinic acetylcholine receptor subunit and receptor subunit protein encoded thereby. Also provided are vectors containing the invention nucleic acids, host cells transformed therewith, alpha9 nicotinic acetylcholine receptor subunit and functional nicotinic acetylcholine receptors comprising at least one alpha9 subunit expressed recombinantly in such host cells as well as transgenic non-human mammals that express the invention receptor subunit and mutants thereof. Receptors of the invention comprise at least one alpha9 nicotinic acetylcholine subunit and form cationic channels activated by acetylcholine, but blocked by nicotine and muscarine. The invention also provides methods for identifying compounds that modulate the ion channel activity of the functional invention receptors containing at least one invention subunit.

13 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Detailed Description Text - DETX:

Vectors employed in the present invention contain both a promoter and a cloning site into which nucleic acid encoding alpha9 receptor subunit(s) can be operatively linked. Such vectors, which are well known in the art, are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potentially inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. (See, for example, Kozak, J. Biol. Chem. 266:19867 (1991)). Similarly, alternative codons, encoding the same amino acid, can be substituted for native codons of the alpha9 nAChR subunit in order to enhance transcription (e.g., the codon preference of the host cell can be adopted, the presence of G-C rich domains can be reduced, and the like).

Detailed Description Text - DETX:

Examples of suitable vectors that may be employed in the present invention include viruses, such as baculoviruses and retroviruses, bacteriophages, cosmids, plasmids and other recombination vehicles typically used in the art. Invention nucleic acids are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic linkers can be ligated to the termini of restricted invention nucleic acids. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA. Additionally, a nucleic acid containing a termination codon and an appropriate restriction site can be ligated into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA. Other means are well known and available in the art.

US-PAT-NO: 5670134

DOCUMENT-IDENTIFIER: US 5670134 A

TITLE: Method for evaluating and modifying biological activity

DATE-ISSUED: September 23, 1997

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|---------------|-------|----------|---------|
| Martin, Jr.; David W. | San Francisco | CA | N/A | N/A |

APPL-NO: 08/ 456330

DATE FILED: June 1, 1995

PARENT-CASE:

This is a continuation of U.S. Ser. No. 08/192,316, filed Feb. 4, 1994, now U.S. Pat. No. 5,470,560, which is a continuation of U.S. Ser. No. 07/947,890 filed Sep. 18, 1992, now abandoned, which is a continuation of U.S. Ser. No. 07/692,806 filed Apr. 25, 1991, now abandoned, which is a continuation of U.S. Ser. No. 07/004,988 filed Jan. 20, 1987, now abandoned.

US-CL-CURRENT: 424/9.2; 424/184.1 ; 424/185.1 ; 424/9.1 ; 435/7.1

ABSTRACT:

Biological effects of agents for diagnostic or therapeutic use are evaluated by administration of the agents to transgenic animals which are transformed with heterologous DNA and which are immune tolerant to the expression product of the heterologous DNA. In a further embodiment, preparations that are immunogenic in the transgenic animal model are purified by reverse immunoaffinity chromatography on antibody obtained from responding transgenic animals.

7 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Drawing Description Text - DRTX:

The nucleic acid encoding the heterologous polypeptide will also contain transcription and translational control sequences recognized by the host.

Typically, these include **promoters** and enhancers to **regulate transcription**, and ribosomal binding domains to regulate translation. Ordinarily, when DNA encoding a human polypeptide is employed it is suitable to use the human transcriptional and translational control domains since they typically are recognized by other mammals. These need not be the control domains of the native gene encoding the heterologous polypeptide. Instead, control domains from other Series, e.g. insulin, are employed in order to confer tissue specificity of expression on the heterologous DNA in the host. However, it is within the scope herein to prepare hybrids comprising the transcriptional and translational control domains native to the host gene-homologous polypeptide ligated to DNA encoding the prepro human protein. Further, the genomic gene encoding the heterologous polypeptide is suitable, notwithstanding that it may contain introns. The introns and their location in the host-homologous gene also can be adapted to the heterologous polypeptide coding domains, as can the host's **codon preference**, including tissue preference domains if the appropriate tissue-specific control domains are used.

Detailed Description Text - DETX:

(e) The pA2.A2 -110 MTV-hGH plasmid was generated from the p-110 MTV-hGH plasmid: two complementary **synthetic DNA oligonucleotides with the sequences**

US-PAT-NO: 5639440

DOCUMENT-IDENTIFIER: US 5639440 A

TITLE: Method for evaluating immunogenicity

DATE-ISSUED: June 17, 1997

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|---------------|-------|----------|---------|
| Martin, Jr.; David W. | San Francisco | CA | 94133 | N/A |

APPL-NO: 08/ 428932

DATE FILED: April 25, 1995

PARENT-CASE:

This is a divisional application of application U.S. Ser. No. 08/192,316, filed Feb. 4, 1994, now U.S. Pat. No. 5,470,560, incorporated herein by reference and to which applications priority is claimed under 35 USC .sctn.120. Application Ser. No. 08,192,316 is a continuation of application Ser. No. 07/947,890, filed Sep. 18, 1992, now abandoned, which is a continuation of application Ser. No. 07/692,806, filed Apr. 25, 1991, now abandoned, which is a continuation of application Ser. No. 08,004,988, filed Jan. 20, 1987, now abandoned.

US-CL-CURRENT: 800/3; 424/184.1 ; 424/9.1 ; 424/9.2 ; 435/7.1

ABSTRACT:

Biological effects of agents for diagnostic or therapeutic use are evaluated by administration of the agents to transgenic animals which are transformed with heterologous DNA and which are immune tolerant to the expression product of the heterologous DNA. In a further embodiment, preparations that are immunogenic in the transgenic animal model are purified by reverse immunoaffinity chromatography on antibody obtained from responding transgenic animals.

11 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

The nucleic acid encoding the heterologous polypeptide will also contain transcription and translational control sequences recognized by the host. Typically, these include **promoters** and enhancers to **regulate transcription**, and ribosomal binding domains to regulate translation. Ordinarily, when DNA encoding a human polypeptide is employed it is suitable to use the human transcriptional and translational control domains since they typically are recognized by other mammals. These need not be the control domains of the native gene encoding the heterologous polypeptide. Instead, control domains from other genes, e.g. insulin, are employed in order to confer tissue specificity of expression on the heterologous DNA in the host. However, it is within the scope herein to prepare hybrids comprising the transcriptional and translational control domains native to the host gene-homologous polypeptide ligated to DNA encoding the prepro human protein. Further, the genomic gene encoding the heterologous polypeptide is suitable, notwithstanding that it may contain introns. The introns and their location in the host-homologous gene also can be adapted to the heterologous polypeptide coding domains, as can the host's **codon preference**, including tissue preference codons if the appropriate tissue-specific control domains are used.

Detailed Description Text - DETX:

(e) The pA2.A2 -110 MTV-hGH plasmid was generated from the p-110 MTV-hGH plasmid: two complementary **synthetic DNA oligonucleotides with the sequences**

US-PAT-NO: 5599907

DOCUMENT-IDENTIFIER: US 5599907 A

TITLE: Production and use of multimeric hemoglobins

DATE-ISSUED: February 4, 1997

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|------------|-------|----------|---------|
| Anderson; David C. | San Bruno | CA | N/A | N/A |
| Mathews; Antony J. | Louisville | CO | N/A | N/A |
| Stetler; Gary L. | Boulder | CO | N/A | N/A |

APPL-NO: 08/ 240712

DATE FILED: May 9, 1994

PARENT-CASE:

This application is a continuation-in-part of Ser. No. 07/789,179, filed Nov. 8, 1991, which is a continuation-in-part of Ser. No. 07/671,707, filed Apr. 1, 1991, now abandoned, which is the national stage of PCT/US90/02654, filed May 10, 1990, which is a continuation-in-part of a Looker and Hoffman, U.S. Ser. No. 07/374,161, DI-ALPHA AND DI-BETA GLOBIN LIKE POLYPEPTIDES AND USES THEREFOR, filed Jun. 30, 1989, now abandoned; (b) Stetler and Wagenbach, U.S. Ser. No. 07/379,116, PRODUCTION OF HUMAN HEMOGLOBIN BY TRANSFORMED YEAST CELLS, filed Jul. 13, 1989, now abandoned; and (c) Hoffman, Looker, Rosendahl and Stetler, U.S. Ser. No. 07/349,623, POLYCISTRONIC CO-EXPRESSION OF THE ALPHA- AND BETA-GLOBINS AND IN VIVO ASSEMBLY OF BIOLOGICALLY ACTIVE, TETRAMERIC HEMOGLOBIN, filed May 10, 1989, now abandoned; all owned by Somatogen, Inc. CROSS-REFERENCE TO RELATED APPLICATIONS Hoffman and Nagai, U.S. Ser. No. 07/194,338, filed May 10, 1988, now U.S. Pat. No. 5,028,588, presently owned by Somatogen, Inc., relates to the use of low oxygen affinity and other mutant hemoglobins as blood substitutes, and to the expression of alpha and beta globin in nonerythroid cells. Hoffman and Nagai, U.S. Ser. No. 07/443,950, filed Dec. 1, 1989, discloses certain additional dicysteine hemoglobin mutants; it is a continuation-in-part of 07/194,338. Anderson, et al., HEMOGLOBINS AS DRUG DELIVERY AGENTS, Ser. No. 07/789,177, filed Nov. 8, 1991, discloses use of conjugation of hemoglobins with drugs as a means for delivery of the drug to a patient.

PCT-DATA:

APPL-NO: PCT/US92/09752
DATE-FILED: November 6, 1992
PUB-NO: WO93/09143
PUB-DATE: May 13, 1993
371-DATE: May 9, 1994
102(E)-DATE: May 9, 1994

US-CL-CURRENT: 530/385; 435/69.1 ; 435/69.7 ; 435/71.1 ; 530/829 ; 536/23.4 ; 536/23.5

ABSTRACT:

Multimeric hemoglobin-like proteins are obtained by crosslinking cysteines of the component tetramers, or by genetically fusing globin-like domains of one tetramer with those of another, by means of an interdomain spacer sequence. Artificial cysteines are introduced selectively in a single globin-like domain per tetramer to control polymerization.

74 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Brief Summary Text - BSTX:

FIG. 2 Shows the sequence [SEQ ID NO:1] of a preferred synthetic gene for expression of (des-Val)-alpha-(Gly)-alpha and des-Val beta globin. This gene is carried by pSGE1.1E4. A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) (SEQ ID NO:25) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The residues are numbered 1 to 141 (SEQ ID NO:26). The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. The residues are numbered 1' to 141' (SEQ ID NO:27). Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. The beta residues are numbered 1 to 146 (SEQ ID NO:28). A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual polypeptide chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially synthetic gene is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in

a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the **promoter** associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the **codon preference** pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong **promoter** constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

This is a derivative of pKK223-3 (Pharmacia LKB, Piscataway, N.J., USA) and pGEM1 (PromegaCorp., Madison, Wis., USA) which carries **synthetic genes** for des-Val alpha globin and des-Val beta globin as part of a polycistronic operon driven by a single Tac promoter.

Other Reference Publication - OREF:

Schoner, B. et al/Expression of Eukaryotic **Genes in Escherichia coli with a Synthetic** Two Cistron System/Methods in Enzymology/vol. 153 Recombinant DNA Part D/Ed: Ray Wu & L. Grossman/Academic Press, Inc./NY/ (1987), 401-416.

US-PAT-NO: 5545727

DOCUMENT-IDENTIFIER: US 5545727 A

TITLE: DNA encoding fused di-alpha globins and production of pseudotetrameric hemoglobin

DATE-ISSUED: August 13, 1996

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-----------|-------|----------|---------|
| Hoffman; Stephen J. | Denver | N/A | N/A | N/A |
| Looker; Douglas L. | Lafayette | CO | N/A | N/A |
| Nagai; Kiyoshi | Cambridge | N/A | N/A | GB2 |

APPL-NO: 07/ 789179

DATE FILED: November 8, 1991

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of application Ser. No. 07/671,707 filed on Apr. 1, 1991, now abandoned, which is a continuation-in-part of PCT/US90/02654, filed on May 10, 1990, which is a continuation-in-part of (a) Ser. No. 07/374,161 filed on Jun. 30, 1989, now abandoned, (b) Ser. No. 07/379,116, filed on Jul. 13, 1989, now abandoned, and (c) Ser. No. 07/349,623, filed on May 10, 1989, now abandoned. All of these prior applications are hereby incorporated by reference. Hoffman and Nagai, U.S. Ser. No. 07/194,338, filed May 10, 1988, now U.S. Pat. No. 5,028,588, presently owned by Somatogen, Inc., relates to the use of low oxygen affinity and other mutant hemoglobins as blood substitutes, and to the expression of alpha and beta globin in nonerythroid cells. Hoffman and Nagai, U.S. Ser. No. 07/443,950, filed Dec. 1, 1989 now pending, discloses certain additional dicysteine hemoglobin mutants; it is a continuation-in-part of Ser. No. 07/194,338 now U.S. Pat. No. 5,028,588. Anderson, et al., HEMOGLOBINS AS DRUG DELIVERY AGENTS Atty. Docket.: U.S. Ser. No. 07/789,177 now abandoned, filed Nov. 8, 1991 now pending, discloses use of conjugation of hemoglobins with drugs as a means for delivery of the drug to a patient.

US-CL-CURRENT: 536/23.4; 530/385 ; 536/23.5

ABSTRACT:

The alpha subunits of hemoglobin, which in nature are formed as separate polypeptide chains which bind noncovalently to the beta subunits, are here provided in the form of the novel molecule di-alpha globin, a single polypeptide chain defined by connecting the two alpha subunits either directly via peptide bond or indirectly by a flexible amino acid or peptide linker. Di-alpha globin may be combined in vivo or in vitro with beta globin and heme

to form hemoglobin. Di-alpha globin is expressed by recombinant DNA techniques. Di-beta globin may be similarly obtained.

DNA encoding alpha globin fusion proteins is provided.

40 Claims, 36 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 70

----- KWIC -----

Brief Summary Text - BSTX:

Saito, et al., J. Biochem., 101: 1281-88 (1987) expressed a **synthetic somatomedin C gene** in E. coli using a two cistron system. They theorized that the instability of somatomedin C, a basic polypeptide, might be overcome by complexing it with an acidic polypeptide. Thus, they constructed a two-cistron system which could express both polypeptides. The termination codon of the first cistron overlapped the initiation codon of the second cistron. The transformants accumulated Somatomedin C at high levels. However, the somatomedin C was recovered in the form of insoluble pellets (see page 1282).

Brief Summary Text - BSTX:

In one embodiment, the alpha- and beta-globin-like polypeptides are co-expressed in bacterial cells. The corresponding **genes may be included in the same synthetic** operon (i.e., driven by one promoter), or placed in separate operons with separate promoters (which may be the same or different). Preferably, expression of the alpha- and beta-globin is enhanced by placing a "ribosomal loader" sequence as hereafter described before each globin gene. This is particularly advantageous in the case of alpha globin which is more difficult to produce in quantity.

Drawing Description Text - DRTX:

FIGS. 4a, 4b and 4c: Oligonucleotides for construction of **synthetic FX-alpha and FX-beta globin genes** (4a to 4c). The top strand is shown 5' to 3' and the bottom strand as 3' to 5'. Areas of overlap between complementary synthetic oligonucleotides are shown as areas where both strands are shown in the same case letters. The PstI site that joins FX-alpha and FX-beta occurs at the overlap of SJH I-35a and SJH I-36b.

Drawing Description Text - DRTX:

FIGS 5a, 5b and 5c: **Synthetic gene** for expression of Met-FX-alpha and Met-FX-beta globin (5a to 5c). Region A contains the alpha globin gene and

region B the beta globin gene. The location of the Factor X sequence and the two Shine-Delgarno sequences (SD#1 and SD#2) in both regions is indicated. Selected restriction sites are also found. The translated amino acid sequences for the ribosomal loader and Met-FX-alpha/and beta-globin are given.

Drawing Description Text - DRTX:

FIGS. 12a, 12b and 12c: Shows the sequence of a preferred synthetic gene for expression of (des-Val)-alpha-(GlyGly)-alpha and des-Val beta globin (12a to 12c). A shows the region (EcoRI to PstI) containing Shine-Delgarno ribosomal binding sites (SD#1 and SD#2), the sequence expressing the octapeptide (Met . . . Glu) which serves as a cotranslational coupler, and the sequence encoding the two nearly identical alpha globin-like polypeptides and the interposed Gly-Gly linker. The first alpha globin sequence begins "Met-Leu", that is, it contains an artifactual methionine, omits the valine which is the normal first residue of mature alpha globin, and continues with the second residue, leucine. The second alpha globin sequence begins "Val-Leu", immediately after the underlined "Gly-Gly" linker. Start and stop codons are underlined. B shows the analogous region (PstI to HindIII) containing the coding sequence for des-Val beta globin. A and B are connected at the PstI site to form a single polycistronic operon.

Detailed Description Text - DETX:

The DNA sequences encoding the individual alpha (or di-alpha) and beta (or di-beta) globin chains may be of genomic, cDNA and synthetic origin, or a combination thereof. Since the genomic globin genes contains introns, genomic DNA must either be expressed in a host which can properly splice the premessenger RNA or modified by excising the introns. Use of an at least partially synthetic gene is preferable for several reasons. First, the codons encoding the desired amino acids may be selected with a view to providing unique or nearly unique restriction sites at convenient points in the sequence, thus facilitating rapid alteration of the sequence by cassette mutagenesis. Second, the codon selection may be made to optimize expression in a selected host. For codon preferences in *E. coli*, see Konigsberg, et al., PNAS, 80:687-91 (1983). For codon preferences in yeast, see the next section. Finally, secondary structures formed by the messenger RNA transcript may interfere with transcription or translation. If so, these secondary structures may be eliminated by altering the codon selections.

Detailed Description Text - DETX:

Intracellular expression of genes in *S. cerevisiae* is primarily affected by the strength of the promoter associated with the gene, the plasmid copy number (for plasmid-borne genes), the transcription terminator, the host strain, and the codon preference pattern of the gene. When secretion of the gene product is desired, the secretion leader sequence becomes significant. It should be noted that with multicopy plasmids, secretion efficiency may be reduced by strong promoter constructions. Ernst, DNA 5:483-491 (1986).

Detailed Description Text - DETX:

The **synthetic FX-beta gene** sequence (included in FIG. 5) was constructed as follows: 100pmole of the following oligo nucleotides were kinased in 3 separate reactions. Reaction 1 contained oligonucleotides SJH I-36b, c, d, e, and f. Reaction 2 contained SJH I-37a, b, c, and e. Reaction 3 contained SJH I-37d, f, and SJH I-38a. After combining the appropriate oligonucleotides, the solutions were lyophilized to dryness and resuspended in 16uL of H₂O. Two uL of 10x kinase buffer (0.5M Tris-HCl, pH7.4, 0.1M MgCl₂), 0.5 uL of 100mM DTT, and 1uL of 1.0mM ATP were then added. The reaction was initiated by addition of 1uL (2U) of T4 polynucleotide kinase (IBI, Inc., New Haven, Conn.). After incubation at 37.degree. C. for 1 hour, the reactions were heated to 95.degree. C. for 10 minutes to inactivate the kinase. The three reactions were combined and 100 pmoles of oligonucleotides SJH I-36a and SJH I-38b were added. After addition of 10uL of 100mM Tris, pH 7.8, 100mM MgCl₂, the oligonucleotides were allowed to anneal by incubating at 65.degree. C. for 30 min, 37.degree. C. for 30min, and 15.degree. C. for 1 hour. Annealed oligonucleotides were ligated by addition of ATP (1mM, final) and DTT (10 mM final) and 4uL (20U) T4 DNA ligase (IBI, Inc., New Haven, Conn.) and incubation at 15.degree. C. for 1 hour. Aliquots of this ligation mixture were then cloned directly into M13mp19 (see below).

Detailed Description Text - DETX:

Other hemoglobin mutants: The **synthetic genes** encoding Hemoglobin Cheverly (beta.sup.45 phe---->ser) Hemoglobin Providence/MSR (beta.sup.82 lys---->asp) and Hemoglobin beta.sup.67 val---->ile and Hemoglobin Kansas (beta.sup.102 asn---->thr) were prepared similarly except with synthetic oligonucleotides spanning the SacII---->BglII, Sall---->SpeI, NcoI---->KpnI and SacI---->SpeI restriction sites respectively (FIG. 7). Synthesis of the mutant oligonucleotides, restriction enzyme digestion, gel purification, and ligation conditions were identical to those used for Hemoglobin Beth Israel. All mutations were first cloned into plasmid pDL II-10a, appropriate clones were sequenced, and the mutated beta globin gene was subcloned into PstI and HindIII digested pDL II-66a. Plasmid sequencing was accomplished as described previously. E. coli cells were transformed, cultured, and induced as previously described. FX-hemoglobin mutants were purified by the method of Example 3. Oxygen binding of purified hemoglobin mutants is shown in Table 9.

Detailed Description Text - DETX:

The recognition site (FX)-encoding sequence could now be removed from pGEM FX-alpha and pGEM FX-beta to obtain pDL II-91f and pDL II-95a, respectively. The des-val alpha globin gene of pDL II-91f was recloned into pKK 223-3 to generate pDL III-1a, the gene being operably linked to the Tac promoter of pKK-223-3. The des-val beta globin gene of pDL II-95a was purified and inserted downstream of the des-val alpha globin gene of pDL III-1a to form a single transcriptional unit which would encode a polycistronic alpha globin/beta globin mRNA, see pDL III-14c. Finally, a **synthetic oligonucleotide**

comprising the desired di-alpha linker encoding sequence and another copy of the alpha globin gene was inserted into pDL III-14c to create pDL III-47a, wherein a Tac promoter controls transcription of a di-alpha globin gene and a des-val beta globin gene.

Detailed Description Text - DETX:

The EagI and PstI restriction fragment containing most of the alpha globin **gene from the plasmid pDL II-91f was gel purified and ligated to a synthetic DNA linker containing the sequence** from the BstBI site of the alpha globin gene to the codon (wild-type Arginine) for its carboxyl terminus, a variable glycine-encoding linker (for example, FIG. 12, RGGV, a di-glycine followed by .alpha. Val; other possibilities include RGM, RGV, RGGV, etc., See Table 200), and the codons for the amino terminal region of alpha globin to the EagI site (FIG. 12). After digesting this ligation mixture with Pst I, the resulting fragment was cloned into BstBI/PstI-cut pDL III-14C to create plasmid pDL III-47a (RGM-di-alpha). Plasmids pDL III-82a (RGGV-di-alpha), pDL IV-Sa (RGV-di-alpha), pDL IV-976 (RV-di-alpha) and pDL IV-66a (RGGGV-di-alpha) were similarly constructed to incorporate the indicated changes in the di-alpha coding sequences.

Detailed Description Text - DETX:

Prior to inserting the globin **genes into the vector it was necessary to incorporate the synthetic translational coupler sequence** into the HpaI site of pPL-lambda-E. This was done by digestion of pPL-lambda-E with HpaI followed by blunt-end ligation of the co-translational coupler into the HpaI site of the vector. Ligation of the coupler to the blunt end resulted in destruction of the HpaI site. The ligation mixture was treated with HpaI to digest any plasmid remaining containing the HpaI site. E. coli N99Ci+ cells were transformed with the resulting reaction mixture. Clones were screened with EcoRI and Hind III restriction digests to identify clones containing the co-translational coupler in the proper orientation. DNA fragments of 522 bp and 4762 bp were observed for plasmid containing the desired orientation. To confirm the orientation of the coupler, the resulting plasmid was sequenced using a primer (5'CAATGGAAAGCAGCAAATCC-3') complementary to the sequence 30 base pairs upstream from the translational coupler sequence. The desired plasmid was denoted as pPL-lambda-E+TC.

Detailed Description Text - DETX:

This **synthetic promoter consists of two functional parts, a regulatory sequence** and sequence that allows efficient initiation of mRNA synthesis. One of the regulatory regions we chose includes the nucleotide sequence that confers positive regulation of transcription in the presence of galactose (M. Johnston and R. Davis, 1984. Molecular and Cellular Biology 4:1440-1448; L. Guarente et al., 1982, Proc Nat Acad Sci (USA) 79:7410-7414.). The transcriptional initiation site is derived from the consensus sequence for the S. cerevisiae glyceraldehyde-3-phosphate dehydrogenase gene (GAP491) (L. McAlister and M. J. Holland, J. Biol Chem 260:15019-15027, 1983; J. P. Holland et al., J. Biol Chem

258:5291-5299, 1983).

Detailed Description Text - DETX:

ASSEMBLY OF THE SYNTHETIC GALACTOSE UPSTREAM ACTIVATOR (GAL.sub.UAS) SEQUENCE

Detailed Description Text - DETX:

The next step in the assembly of this hybrid promoter was to clone the SphI - Sall fragment containing the GAL.sub.UAS into pGS2888. pGS2888 was digested with SphI and Sall, phenol-chloroform extracted and ethanol precipitated. Fifty nanograms of SphI, Sall digested pGS2888 was incubated with 25ng of the annealed, ligated GAL.sub.UAS mixture in 0.005ml 1.times. ligase buffer containing 10 units of T4 DNA ligase. The ligation mixture was incubated overnight at 4.degree. C. and a portion used to transform E. coli DH5.alpha.. Ampicillin resistant clones were isolated and plasmid DNA prepared. The plasmid DNA (digested with XbaI and SphI) was analyzed by agarose gel electrophoresis. A plasmid containing a fragment of the expected size (.sup..about. 500bp) was identified. The sequence of the putative GAL.sub.UAS portion of this plasmid was determined and the plasmid was designated pGS4788 (FIG. 21(b)). The complete sequence of the synthetic GALGAP promoter (pGGAP) is shown in FIG. 20.

Detailed Description Text - DETX:

Removal of the PstI and SpeI sites from pGS4888. The design of the synthetic linker for joining two .alpha.-globin chains allows the inclusion of PstI and SpeI sites flanking a 30bp sequence that includes the junction of the two .alpha.-globin coding sequences. Because we anticipate testing several different linker sequences, these sites will allow directional cloning of relatively short synthetic oligonucleotides encoding different linker sequences. Removal of the PstI and SpeI sites from the vector sequence is, therefore, necessary so that the sites in the coding region are usable. One .mu.g of the plasmid pGS4888 was digested with PstI and ethanol precipitated. The dry pellet was resuspended in 50 .mu.l of 33mM Tris-acetate, pH7.9, 66mM potassium acetate, 10mM magnesium acetate, 0.5mM DTT and 50 .mu.M of each dNTP (T4 polymerase buffer). Two units of T4 DNA polymerase were added and the reaction mixture incubated for 15min at 37.degree. C. Na.sub.3 EDTA was added to 12.5mM and the reaction mixture heated to 65.degree. C. for 15min, phenol extracted and ethanol precipitated. The dry pellet was dissolved in 14 .mu.l of T4 DNA ligase buffer (BRL) and 1 .mu.l (10 units) of DNA ligase added. The ligation mixture was incubated a 4.degree. C. for 16hr. A portion of the ligation reaction was used to transform E. coli DH5.alpha. and transformants were selected on LB-ampicillin plates. Plasmid DNA was prepared from 12 transformants. The DNA was analyzed by agarose gel electrophoresis of PstI digests. Five transformants had lost the PstI site and one of these was designated pGS1889. The SpeI site of this plasmid was removed as described above after digestion of pGS1889 with SpeI. A plasmid was identified that had lost both the PstI and the SpeI site and was designated pGS1989.

Detailed Description Text - DETX:

Parental plasmid is pSGE224 (42) The linker sequences are synthetic DNA.

Other Reference Publication - OREF:

Schulz et al., "Increased Expression in E. coli of a Synthetic Gene . . ." J. Bacteriology 169(12):5385-5392, Dec. 1987.

Other Reference Publication - OREF:

Abstract DBA Accession No. 89-12842 on Dialog File 357, of DeBaetelier, et al.; Yeast as a source of human lysozyme--synthetic gene cloning and expression in S. cerevisiae, Medec. Fac. Landbouwet. Rijksuniv. Gent, 53:2135-2141 (1988).

US-PAT-NO: 5470560

DOCUMENT-IDENTIFIER: US 5470560 A

TITLE: Method for evaluating immunogenicity

DATE-ISSUED: November 28, 1995

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|---------------|-------|----------|---------|
| Martin, Jr.; David W. | San Francisco | CA | N/A | N/A |

APPL-NO: 08/ 192316

DATE FILED: February 4, 1994

PARENT-CASE:

This is a continuation of U.S. Ser. No. 07/947,890 filed Sep. 18, 1992, now abandoned, which is a continuation of U.S. Ser. No. 07/692,806 filed Apr., 25 1991, now abandoned which is a continuation of U.S. Ser. No. 07/004,988 filed Jan. 20, 1987, now abandoned.

US-CL-CURRENT: 435/6; 424/184.1 ; 424/9.1 ; 435/7.1

ABSTRACT:

Biological effects of agents for diagnostic or therapeutic use are evaluated by administration of the agents to transgenic animals which are transformed with heterologous DNA and which are immune tolerant to the expression product of the heterologous DNA. In a further embodiment, preparations that are immunogenic in the transgenic animal model are purified by reverse immunoaffinity chromatography on antibody obtained from responding transgenic animals.

9 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

The nucleic acid encoding the heterologous polypeptide will also contain transcription and translational control sequences recognized by the host. Typically, these include promoters and enhancers to regulate transcription, and

ribosomal binding domains to regulate translation. Ordinarily, when DNA encoding a human polypeptide is employed it is suitable to use the human transcriptional and translational control domains since they typically are recognized by other mammals. These need not be the control domains of the native gene encoding the heterologous polypeptide. Instead, control domains from other genes, e.g. insulin, are employed in order to confer tissue specificity of expression on the heterologous DNA in the host. However, it is within the scope herein to prepare hybrids comprising the transcriptional and translational control domains native to the host gene-homologous polypeptide ligated to DNA encoding the prepro human protein. Further, the genomic gene encoding the heterologous polypeptide is suitable, notwithstanding that it may contain introns. The introns and their location in the host-homologous gene also can be adapted to the heterologous polypeptide coding domains, as can the host's codon preference, including tissue preference codons if the appropriate tissue-specific control domains are used.

Detailed Description Text - DETX:

(e) The pA2.A2 -110 MTV-hGH plasmid was generated from the p-110 MTV-hGH plasmid: two complementary synthetic DNA oligonucleotides with the sequences

| | L # | Hits | Search Text | DBs | Time Stamp |
|----|-----|-------|--|--------------------|---------------------|
| 1 | L1 | 22789 | (humaniz\$ or synthetic) near8 (gene\$1 or sequence\$1) | USPAT; US-PGPUB | 2003/01/31 11:57 |
| 2 | L2 | 2753 | 1 same (regulat\$8 or codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:10 |
| 3 | L3 | 4647 | 1 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 4 | L4 | 1411 | 2 and 3 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 5 | L5 | 966 | 2 same muta\$10 | USPAT; US-PGPUB | 2003/01/31 12:00 |
| 6 | L6 | 4 | 1 same (regulat\$8 and codon adj preference) | USPAT; US-PGPUB | 2003/01/31 12:02 |
| 7 | L7 | 144 | (transcription near2 (regulat\$8 or factor\$1) or splice adj site\$1 or promoter\$1 or addition adj site\$1) same codon adj preference | USPAT; US-PGPUB | 2003/01/31 12:13 |
| 8 | L8 | 116 | 7 and 1 | USPAT; US-PGPUB | 2003/01/31 14:41 |
| 9 | L9 | 208 | 1 same (luciferase\$1 or gfp) | USPAT; US-PGPUB | 2003/01/31 14:42 |
| 10 | L10 | 30 | 4 and 9 | USPAT; US-PGPUB | 2003/01/31 14:42 |

PGPUB-DOCUMENT-NUMBER: 20030024006

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030024006 A1

TITLE: Gene switches

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|-----------|-------|---------|---------|
| Choo, Yen | Cambridge | | GB | |
| Ullman, Christopher | London | | GB | |
| Graeme | | | | |

APPL-NO: 09/ 995973

DATE FILED: November 28, 2001

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|----------------|-----------------------|------------------|
| GB | PCT/GB00/02071 | 2000GB-PCT/GB00/02071 | May 30, 2000 |
| GB | 9912635.1 | 1999GB-9912635.1 | May 28, 1999 |
| GB | 0001578.4 | 2000GB-0001578.4 | January 24, 2000 |

US-CL-CURRENT: 800/278

ABSTRACT:

Disclosed herein are methods and compositions relating to gene switches that use molecule capable of binding DNA sequences.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0200] Any changes that are made to the coding sequence can be made using techniques that are well known in the art and include site directed **mutagenesis**, PCR, and **synthetic gene** construction. Such methods are described in published patent applications EP 0 385 962 (to Monsanto), EP 0 359 472 (to Lubrizol) and WO 93/07278 (to Ciba-Geigy). Well known protocols for transient expression in plants can be used to check the expression of modified genes before their transfer to plants by transformation.

Detail Description Paragraph - DETX:

[0437] To investigate the utility of heterologous zinc finger proteins for the regulation of plant genes, a synthetic zinc finger protein was designed and introduced into transgenic *Arabidopsis thaliana* under the control of a promoter capable of expression in a plant as described below. A second construct comprising the zinc finger protein binding sequence fused upstream of the Green Fluorescent Protein (GFP) reporter gene was also introduced into transgenic *Arabidopsis thaliana* as described in Example 8. Crossing the two transgenic lines produced progeny plants carrying both constructs in which the GFP reporter gene was expressed demonstrating transactivation of the gene by the zinc finger protein.

PGPUB-DOCUMENT-NUMBER: 20020192185

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020192185 A1

TITLE: Recombinant protein production in bovine adenovirus expression vector system

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|----------------|-------|---------|---------|
| Mittal, Suresh K. | West Lafayette | IN | US | |
| Graham, Frank L. | Rome | | IT | |
| Prevec, Ludvik | Burlington | | CA | |
| Babiuk, Lorne A. | Saskatoon | | CA | |

APPL-NO: 10/ 046938

DATE FILED: January 14, 2002

RELATED-US-APPL-DATA:

child 10046938 A1 20020114 parent continuation-of 09435242 19991105 US GRANTED
parent-patent 6379944 US child 09435242 19991105 US parent continuation-of
08815927 19970313 US GRANTED parent-patent 6086890 US child 08815927 19970313
US parent continuation-of 08164292 19931209 US GRANTED parent-patent 5820868 US

US-CL-CURRENT: 424/93.2,424/199.1 ,424/233.1 ,435/235.1 ,435/320.1 ,435/456

ABSTRACT:

The present invention relates novel live bovine adenovirus (BAV) expression vector systems in which part or all of one or both of the early region 1 (E1) and early region 3 (E3) genes are deleted and replaced by a foreign gene or fragment thereof and novel recombinant mammalian cell lines stably transformed with BAV E1 sequences, and therefore, express E1 gene products capable of allowing replication therein of a bovine adenovirus having an E1 deletion replaced by a heterologous nucleotide sequence encoding a foreign gene or fragment thereof and their use in production of (antigenic) polypeptides or fragments thereof for the purpose of live recombinant virus or subunit vaccine or for other therapies.

----- KWIC -----

Detail Description Paragraph - DETX:

[0053] A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate **regulatory** sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA **sequences from eucaryotic (e.g., mammalian) DNA, viral DNA, and even synthetic DNA sequences.** A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Detail Description Paragraph - DETX:

[0063] A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a viral gene, the gene will usually be flanked by DNA that does not flank the viral gene in the genome of the source virus or virus-infected cells. Another example of the heterologous coding **sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences** having codons different from the native gene). Allelic variation or naturally occurring **mutational** events do not give rise to a heterologous region of DNA, as used herein.

Detail Description Paragraph - DETX:

[0132] **Luciferase** was expressed as an active enzyme as determined by **luciferase** assays using extracts from MDBK cells-infected with-BAV3-Luc (see FIG. 13). The **luciferase** gene without any exogenous **regulatory** sequences was inserted into E3 of the BAV3 genome, therefore, there was a possibility of **luciferase** expression as a fusion protein with part of an E3 protein if the **luciferase** gene was in the same frame, Such as, F1 and F3 which represent open reading frames (ORFs) for E3 proteins (FIG. 15) or the fusion protein may arise due to recognition of an upstream initiation codon in the **luciferase** ORF. To explore this possibility we sequenced the DNA at the junction of the **luciferase gene and the BAV3 sequences with the help of a plasmid, pSM51-Luc and a synthetic primer design to bind luciferase coding sequences** near the initiation codon (data not shown).

PGPUB-DOCUMENT-NUMBER: 20020168707

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168707 A1

TITLE: SYNTHETIC GENES AND GENETIC CONSTRUCTS COMPRISING SAME I

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|------|-----------|---------|---------|
| GRAHAM, MICHAEL WAYNE | | ST. LUCIA | | AU |

APPL-NO: 09/ 100812

DATE FILED: June 19, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|---------|---------------|----------------|
| AU | PP2292 | 1998AU-PP2292 | March 20, 1998 |

US-CL-CURRENT: 435/69.1,424/93.2 ,435/320.1 ,435/325 ,435/455 ,514/44 ,536/23.1 ,536/23.5

ABSTRACT:

The present invention relates generally to synthetic genes for modifying endogenous gene expression in a cell, tissue or organ of a transgenic organism, in particular a transgenic animal or plant. More particularly, the present invention provides novel synthetic genes and genetic constructs which are capable of repressing delaying or otherwise reducing the expression of an endogenous gene or a target gene in an organism when introduced thereto.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0005] In work leading up to the present invention, the inventors sought to elucidate the mechanisms involved in down-regulating gene expression in an attempt to provide improved methods therefor. In so doing the inventors have developed a wide range of synthetic genes capable of modulating gene expression in both prokaryotic and eukaryotic cells and genetic constructs comprising same.

Detail Description Paragraph - DETX:

[0049] The term "**synthetic gene**" refers to a non-naturally occurring gene as hereinbefore defined which preferably comprises at least one or more transcriptional and/or translational **regulatory** sequences operably linked to a structural gene sequence.

Detail Description Paragraph - DETX:

[0057] Generally, a gene of the invention may be subjected to **mutagenesis** to produce single or multiple nucleotide substitutions, deletions and/or additions without affecting its ability to modify target gene expression. Nucleotide insertional derivatives of the **synthetic gene** of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product.

Detail Description Paragraph - DETX:

[0067] Alternatively, the structural gene may comprise a nucleotide sequence which does not encode an amino acid sequence or more commonly, comprises one or more open reading frames which encode one or more peptides, oligopeptides or polypeptides which are unrelated at the amino acid sequence level to the amino acid sequence encoded by the target gene. For example, the mRNA product of the structural **gene may be inserted into the synthetic gene** of the invention so as to alter or disrupt the reading frame of the structural gene and produce one or more frame shift **mutations** in the translation product thereof relative to the translation product encoded by the target gene, notwithstanding a substantial identity between the structural gene and the target gene on the one hand and the corresponding mRNA products of the structural gene and the target gene on the other hand. Such effects may be produced by introducing one or two nucleotide substitutions or deletions into the structural gene, relative to the target gene sequence or alternatively, by introducing a translation start codon 5'-ATG-3' upstream of any nucleotide in the structural gene which occurs at a particular position in a codon of the corresponding target gene such that the position of said nucleotide in the codon of the structural gene is altered.

Detail Description Paragraph - DETX:

[0071] A promoter is usually, but not necessarily, positioned upstream or 5', of the structural **gene component of the synthetic gene** of the invention, the expression of which it **regulates**. Furthermore, the **regulatory** elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the structural gene.

Detail Description Paragraph - DETX:

[0074] Examples of promoters suitable for use in the **synthetic genes** of the present invention include viral, fungal, bacterial, animal and plant derived promoters capable of functioning in plant, animal, insect, fungal, yeast or bacterial cells. The promoter may **regulate** the expression of the structural gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or pathogens, or metal ions, amongst others.

Detail Description Paragraph - DETX:

Synthetic Genes and Genetic Constructs Comprising the lacI and Green Fluorescent Protein (GFP) Open **Reading** Frames

Detail Description Paragraph - DETX:

Synthetic Genes and Genetic Constructs Comprising the lacI and Green Fluorescent Protein (GFP) and **Tyrosinase** Open Reading Frames

PGPUB-DOCUMENT-NUMBER: 20020064842

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020064842 A1

TITLE: Renilla reniformis green fluorescent protein and mutants thereof

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------------|-----------|-------|---------|---------|
| Sorge, Joseph A. | Wilson | WY | US | |
| Vaillancourt, Peter E. | Del Mar | CA | US | |
| Felts, Katherine A. | San Diego | CA | US | |

APPL-NO: 09/ 795040

DATE FILED: February 26, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60210561 20000609 US

US-CL-CURRENT: 435/183,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

The invention relates to recombinant polynucleotides encoding the Green Fluorescent Protein (GFP) from *R. reniformis*, as well as polynucleotides encoding variants and fusion polypeptides of *R. reniformis* GFP. The invention further relates to vectors encoding *R. Reniformis* GFP and variants and fusions thereof, as well as to cells comprising and/or expressing such vectors. The invention also relates to recombinant *R. reniformis* GFP polypeptides and fusion polypeptides and variants thereof, as well as to methods of making and using such polypeptides both in vivo and in vitro.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0047] The invention further encompasses a polynucleotide encoding *R. reniformis* **GFP** or a variant of *R. reniformis* **GFP**, wherein the polynucleotide comprises at least one **humanized codon sequence**.

Summary of Invention Paragraph - BSTX:

[0053] The term "variant thereof" when used in reference to an R. reniformis polynucleotide coding sequence means that the sequence bears one or more nucleotide differences relative to the sequence of the wild-type R. reniformis coding sequence. A variant of an R. reniformis polynucleotide sequence encodes an R. reniformis **GFP** polypeptide or a variant thereof. A variant of an R. reniformis polynucleotide coding **sequence includes a humanized polynucleotide coding sequence**. A variant polynucleotide directs the expression of an amount of fluorescent polypeptide at least equal to, or greater than, the amount expressed from an equal mass amount or from an equal number of copies of a non-**humanized R. reniformis GFP polynucleotide sequence**.

Summary of Invention Paragraph - BSTX:

[0054] The term "**humanized polynucleotide**" or "**humanized sequence**" refers to a polynucleotide coding sequence in which one or more, including 5 or more, 10 or more, 20 or more, 50 or more, 75 or more, 100 or more, 125 or more, 150 or more, 200 or more, or even all codons of the polynucleotide coding sequence for a non-human polypeptide (i.e., a polypeptide not naturally expressed in humans) have been altered to a codon sequence more preferred for expression in human cells. Because there are 64 possible combinations of the 4 DNA nucleotides in codon groups of 3, the genetic code is redundant for many of the 20 amino acids. Each of the different codons for a given amino acid encodes the incorporation of that amino acid into a polypeptide. However, within a given species there tends to be a preference for certain of the redundant codons to encode a given amino acid. The "**codon preference**" of R. reniformis is different from that of humans (this **codon preference** is usually based upon differences in the level of expression of the tRNAs containing the corresponding anticodon sequences). In order to obtain high expression of a non-human gene product in human cells, it is advantageous to change one or more non-preferred codons to a codon sequence that is preferred in human cells. Table 1 shows the preferred codons for human gene expression. A codon sequence is preferred for human expression if it occurs to the left of a given codon sequence in the table. Optimally, but not necessarily, less preferred codons in a non-human polynucleotide coding **sequence are humanized by altering them to the codon most preferred for that amino acid in human gene** expression. The amount of fluorescent polypeptide expressed in a human cell from a **humanized GFP polynucleotide sequence** is at least two-fold greater, on either a mass or a fluorescence intensity scale per cell, than the amount expressed from an equal amount or number of copies of a non-humanized **GFP** polynucleotide.

Summary of Invention Paragraph - BSTX:

[0055] As used herein, the term "**humanized codon**" means a **codon sequence**, within a polynucleotide sequence encoding a non-human polypeptide, that has been changed to a codon sequence that is more preferred for expression in human cells relative to that codon encoded by the non-human organism from which the non-human polypeptide is derived. Species-specific codon preferences stem in part from differences in the expression of tRNA molecules with the appropriate anticodon sequence. That is, one factor in the species-specific **codon preference** is the relationship between a codon and the amount of corresponding

anticodon tRNA expressed.

Summary of Invention Paragraph - BSTX:

[0056] It should be understood that any of the recombinant vectors of the invention may comprise a humanized polynucleotide encoding R. reniformis **GFP** or a variant thereof. Similarly, any of the cells of the invention may comprise vectors comprising a humanized polynucleotide encoding R. reniformis **GFP** or a variant thereof. It should also be understood that all claimed methods using polynucleotides encoding R. reniformis **GFP** may be performed with humanized polynucleotides encoding R. reniformis **GFP** or variants of R. reniformis **GFP**. Finally, any R. reniformis **GFP** polypeptide of the invention may be expressed from a **humanized R. reniformis GFP polynucleotide coding sequence**.

Brief Description of Drawings Paragraph - DRTX:

[0081] FIG. 5 shows the **sequence of a humanized R. reniformis GFP polynucleotide sequence** (SEQ ID NO: 3).

Brief Description of Drawings Paragraph - DRTX:

[0082] FIG. 6 shows a **sequence alignment between non-humanized** and humanized R. reniformis **GFP**. Vertical lines represent homology between the **humanized and non-humanized genes**. Gaps represent nucleotides that were altered to produce the hrGFP gene.

Detail Description Paragraph - DETX:

[0229] The **humanized recombinant GFP (hrGFP) nucleotide sequence** was altered according to Haas, J. et. al., 1996, Curr. Biol. 6[3]:315-324, such that all the codons were selected based on their prevalence in genes that are highly expressed in human cells. The sequence is set forth in SEQ ID NO: 3 (see FIG. 5). FIG. 6 shows a **sequence alignment of the non-humanized** recombinant R. reniformis **GFP** (SEQ ID NO: 1) and **humanized R. reniformis GFP polynucleotide sequences**. The **humanized gene** was constructed by synthesizing a set of complementary, overlapping oligonucleotides which were annealed, ligated and subcloned. Both strands were completely sequenced, and **mutations** were corrected using the QuickChange kit (Stratagene). The PCR fragment was digested to completion with EcoR I and Xho I and inserted between the EcoR I and Xho I sites of the retroviral expression vector pFB (Stratagene) to create the vector pFB-hrGFP. This vector was used for further analysis of the **humanized gene**.

Detail Description Paragraph - DETX:

[0231] Virus production was carried out by co-transfecting 293T cells with 3 .mu.g each of the vectors pGPhisD (Stratagene), pVSV-G-puro (Stratagene), and

either pFB-hrGFP or the vector pFB-EGFP. The latter vector contains a copy of the fully **humanized, redshifted A. victoria GFP gene** (EGFP). The vectors pGPhisD and pVSV-G-puro encode the viral proteins gag-pol and VSV-G, which are required in trans for production of virus. The transfections were carried out using the MBS Transfection Kit (Stratagene), with some modifications. For each transfection, 2.5.times.10.sup.6 293T cells were plated in a 60 mm tissue culture dish. The following day medium was aspirated and replaced with 4 ml pre-warmed DMEM supplemented with 7% MBS and 25 .mu.M chloroquine (Sigma, St. Louis, Mo.) prior to transfection. The DNA/CaPO.sub.4 transfection mixes were prepared according to the manufacture's recommended protocol and added to the cells. After a 3 h incubation, the medium was replaced with 4 ml of pre-warmed complete culture medium (DMEM containing 10% FBS) supplemented with 25 .mu.M chloroquine and incubated for 6-7 hours. The medium was then replaced with 4 ml pre-warmed DMEM+10% FBS. Cells were incubated overnight (12-16 hours), and medium was replaced with 3 ml pre-warmed DMEM+10% FBS, and virus was collected overnight (24 hours). The 3 ml viral supernatant was removed and filtered through a 0.45 .mu.m filter. Supernatants were stored on ice for immediate use or frozen on dry ice and stored at -80.degree. C.

Detail Description Paragraph - DETX:

Evaluation of the Expression of R. reniformis **GFP from a Humanized Polynucleotide Sequence**

Detail Description Paragraph - DETX:

[0232] The **humanized R. reniformis GFP coding sequence** described in Example 5 has been tested for expression in several human, rodent and monkey cell lines. Fluorescence levels have been found to be substantially higher for the **humanized rGFP (hrGFP) gene** compared with that for rGFP. In a direct comparison between cell populations harboring single copy proviral expression cassettes encoding either hrGFP or the humanized, red-shifted Aequorea **GFP** (EGFP), we found relative fluorescence intensity to be comparable between the two genes. Viral Transduction. One day prior to transduction, 293 cells (human) or CHO cells (hamster) were plated in DMEM supplemented with 10% FBS at 1.times.10.sup.5 cells/well in a 6 well tissue culture dish. The following day the viral supernatants were serially diluted in DMEM+10% FBS to a final volume of 1.0 ml/sample, and supplemented with DEAE-Dextran (Sigma, St. Louis, Mo., catalog #D-9885) to a final concentration of 10 .mu.g/ml. Culture medium was removed from the target cells and replaced with 1 ml of viral dilution. Each diluted viral sample was applied to a well containing the target cells, and incubated for 3 h, after which 1 ml of pre-warmed DMEM+10% FBS was added to each well, and the plates were then incubated for 2 d. After 2 d the plates were washed 2.times. with PBS, trypsinized, pelleted by centrifugation, and resuspended in 1.0 ml PBS. Cell suspensions were stored on ice and analyzed by Fluorescence Activated Cell Sorting (FACS) within one hour. FACS analysis was performed by Cytometry Research Services, (Sorrento Valley, Calif.).

Claims Text - CLTX:

37. A polynucleotide encoding *R. reniformis* **GFP** or a variant of *R. reniformis* **GFP**, wherein said polynucleotide comprises at least one **humanized codon sequence**.

PGPUB-DOCUMENT-NUMBER: 20020046419

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020046419 A1

TITLE: Regulated gene expression in plants

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|-----------|-------|---------|---------|
| Choo, Yen | Cambridge | NY | GB | |
| Ullman, Christopher | London | NY | GB | |
| Graeme | Scarsdale | | US | |
| Chua, Nam-Hai | New York | | US | |
| Sanchez, Juan Pablo | | | | |

APPL-NO: 09/ 732348

DATE FILED: December 7, 2000

RELATED-US-APPL-DATA:

child 09732348 A1 20001207 parent continuation-in-part-of PCT/GB00/02071
20000530 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|-----------|------------------|------------------|
| GB | 9912635.1 | 1999GB-9912635.1 | May 18, 1999 |
| GB | 001578.4 | 2000GB-001578.4 | January 24, 2000 |

US-CL-CURRENT: 800/298,435/419 ,800/278

ABSTRACT:

A method is provided of regulating transcription in a plant cell from a DNA sequence comprising a target DNA operably linked to a coding sequence, which method comprises introducing an engineered zinc finger polypeptide in said plant cell which polypeptide binds to the target DNA and modulates transcription of the coding sequence.

REFERENCE TO RELATED APPLICATIONS/INCORPORATION BY REFERENCE
STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED
RESEARCH

[0001] This application is a continuation-in-part of PCT application no. PCT/GB00/02071 entitled "GENE SWITCHES" filed May 30, 2000 designating the U.S. and claiming priority from GB applications 9912635.1 filed May 18, 1999 and 001578.4 filed Jan. 24, 2000. Further mentioned and incorporated by reference

herein are PCT/GB99/03730, filed Nov. 9, 1999, published as WO00/27878A1 on May 18, 2000 entitled "Screening System For Zinc Finger Polypeptides For A Desired Binding Ability" and claiming priority from GB application 9824544.2, filed Nov. 9, 1998, and designating the U.S.; PCT/GB99/03730 which is a continuation-in-part of U.S. patent application Ser. No. 09/139,672, filed Aug. 25, 1998 (now U.S. Pat. No. 6,013,453), which is a continuation of U.S. patent application Ser. No. 08/793,408 (now U.S. Pat. No. 6,007,988), filed as PCT application No. PCT/GB95/01949 on Aug. 17, 1995, designating the U.S. and, published as WO96/06166 on Feb. 29, 1996 entitled "Improvements in or Relating to Binding Proteins for Recognition of DNA"; PCT/GB95/01949 claims the benefit of priority from GB application 9514698.1, filed Jul. 19, 1995, GB application 9422534.9, filed Nov. 8, 1994 and GB application No. 9416880.4, filed Aug. 20, 1994. Mention is also made of: U.S. Ser. No. 08/422,107; WO96/32475; WO99/47656A2, published Sep. 23, 1999 entitled "Nucleic Acid Binding Proteins"; WO98/53060A1, published Nov. 26, 1998 entitled "Nucleic Acid Binding Proteins"; WO98/53059A1 published Nov. 26, 1998 entitled "Nucleic Acid Binding Proteins"; WO98/53058A1 published Nov. 26, 1998 entitled "Nucleic Acid Binding Proteins"; WO98/53057A1 published Nov. 26, 1998 ("Nucleic Acid Binding Polypeptide Library"; U.S. Pat. Nos. 6,013,453 and 6,007,988; Fiehn et al. (2000) Nature Biotechnol. 18:1157-1161; Richter et al. (2000) Nature Biotechnol. 18:1167-1171; and, generally, Nature Biotechnol. Vol. 18(11) together with all documents cited or referenced therein. Each of the foregoing applications and patents, and each document cited or referenced in each of the foregoing applications and patents, including during the prosecution of each of the foregoing applications and patents ("application cited documents") and any manufacturer's instructions or catalogues for any products cited or mentioned in each of the foregoing applications and patents and in any of the application cited documents, are hereby incorporated herein by reference. Furthermore, all documents cited in this text, and all documents cited or referenced in documents cited in this text, and any manufacturer's instructions or catalogues for any products cited or mentioned in this text, are hereby incorporated herein by reference.

[0002] Not applicable.

----- KWIC -----

Detail Description Paragraph - DETX:

[0163] Any changes that are made to the coding sequence can be made using techniques that are well known in the art and include site directed **mutagenesis**, PCR, and **synthetic gene** construction. Such methods are described in published patent applications EP 0 385 962 , EP 0 359 472 and WO93/07278. Well-known protocols for transient expression in plants can be used to check the expression of modified genes before their transfer to plants by transformation.

Detail Description Paragraph - DETX:

[0218] To investigate the utility of heterologous zinc finger proteins for the **regulation** of plant **genes**, a **synthetic** zinc finger protein was designed and

introduced into transgenic *A. thaliana* under the control of a promoter capable of expression in a plant as described below. A second construct comprising the zinc finger protein binding sequence fused upstream of the Green Fluorescent Protein (**GFP**) reporter gene was also introduced into transgenic *A. thaliana* as described in Example 2. Crossing the two transgenic lines produced progeny plants carrying both constructs in which the **GFP** reporter gene was expressed demonstrating transactivation of the gene by the zinc finger protein.

PGPUB-DOCUMENT-NUMBER: 20020034757

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020034757 A1

TITLE: Single-molecule selection methods and compositions therefrom

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|-----------|-------|---------|---------|
| Cubicciotti, Roger S. | Montclair | NJ | US | |

APPL-NO: 09/ 907385

DATE FILED: July 17, 2001

RELATED-US-APPL-DATA:

child 09907385 A1 20010717 parent continuation-of 09081930 19980520 US GRANTED
parent-patent 6287765 US

US-CL-CURRENT: 435/6,435/91.2 ,536/22.1 ,536/23.1 ,536/24.3

ABSTRACT:

Single-molecule selection methods are provided for identifying target-binding molecules from diverse sequence and shape libraries. Complexes and imprints of selected target-binding molecules are also provided. The subject selection methods are used to identify oligonucleotide and nonnucleotide molecules with desirable properties for use in pharmaceuticals, drug discovery, drug delivery, diagnostics, medical devices, cosmetics, agriculture, environmental remediation, smart materials, packaging, microelectronics and nanofabrication. Single oligonucleotide molecules with desirable binding properties are selected from diverse sequence libraries and identified by amplification and sequencing. Alternatively, selected oligonucleotide molecules are identified by sequencing without amplification. Nonnucleotide molecules with desirable properties are identified by single-molecule selection from libraries of conjugated molecules or nucleotide-encoded nonnucleotide molecules. Alternatively, target-specific nonnucleotide molecules are prepared by imprinting selected oligonucleotide molecules into nonnucleotide molecular media. Complexes and imprints of molecules identified by single-molecule selection are shown to have broad utility as drugs, prodrugs, drug delivery systems, willfully reversible cosmetics, diagnostic reagents, sensors, transducers, actuators, adhesives, adherents and novel multimolecular devices.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0083] The term "conjugate" means two or more molecules, at least one being a selected molecule, attached to one another in an irreversible or pseudoirreversible manner, typically by covalent and/or specific attachment. A first selected molecule may be conjugated to a second molecule or to a nucleic acid sequence either indirectly, e.g., through an intervening spacer arm, group, molecule, bridge, carrier, or specific recognition partner, or directly, i.e., without an intervening spacer arm, group, molecule, bridge, carrier or specific recognition partner, advantageously by direct covalent attachment. A selected molecule may be conjugated to a nucleotide via hybridization, provided the selected molecule is tagged with an oligonucleotide complementary to a selected nucleic acid sequence comprising the nucleotide. Other noncovalent means for conjugation of nucleotide and nonnucleotide molecules include, e.g., ionic bonding, hydrophobic interactions, ligand-nucleotide binding, chelating agent/metal ion pairs or specific binding pairs such as avidin/biotin, streptavidin/biotin, anti-fluorescein/fluorescein, anti-2,4-dinitrophenol (DNP)/DNP, anti-peroxidase/oxidase, anti-digoxigenin/digoxigenin or, more generally, receptor/ligand. For example, a reporter molecule such as alkaline phosphatase, horseradish peroxidase, 13-galactosidase, urease, luciferase, rhodamine, fluorescein, phycoerythrin, luminol, isoluminol, an acridinium ester or a fluorescent microsphere which is attached, e.g., for labeling purposes, to a selected molecule or selected nucleic acid sequence using avidin/biotin, streptavidin/biotin, anti-fluorescein/fluorescein, anti-peroxidase/oxidase, anti-DNP/DNP, anti-digoxigenin/digoxigenin or receptor/ligand (i.e., rather than being directly and covalently attached) is said to be conjugated to the selected molecule or selected nucleic acid sequence by means of a specific binding pair. The term "conjugate" does not include an unmodified sequence of nucleotides, referred to herein as a molecule, nucleic acid, nucleotide, defined sequence segment, nucleotide sequence or oligonucleotide. However, oligonucleotides, aptamers, synthetic heteropolymers, defined sequence segments and selected nucleic acid sequences may be referred to as conjugates if a nonnucleotide molecule, group or moiety (e.g., biotin, digoxigenin, fluorescein, rhodamine) is introduced as a nucleotide analog, modified nucleotide or nucleoside triphosphate before, during or after nucleic acid synthesis.

Summary of Invention Paragraph - BSTX:

[0265] "Selected nucleic acid sequences" include, but are not limited to, defined sequence segments of synthetic heteropolymers and discrete structures, heteropolymeric, aptameric and nonaptameric nucleotide-based devices, oligonucleotides, and RNA, DNA or denatured RNA or DNA sequences, including wild-type, mutant and recombinant biological nucleic acid sequences; biological, recombinant, engineered and synthetic nucleic acids comprising specific or catalytic recognition sites or properties, e.g., aptamers, catalytic DNA, ribozymes, nucleic acid ligands, nucleic acid receptors, nucleic acid antibodies and nucleic acid molecules capable of participating in specific recognition, catalytic and enzymatic reactions; genomic, plasmid, cellular and transcribed or complementary nucleic acids, including DNA, cDNA and RNA; natural and synthetic coding, noncoding, initiation, termination, promoter and

regulatory sequences, including natural, synthetic, native or nonnative biological recognition sites and therapeutic targets; natural and synthetic oligonucleotides with defined topology, secondary or tertiary structure or three-dimensional shape, including rolling and circular nucleic acids, nucleic acid loops, stems, bulges, knots, pseudoknots, polygons, spheres, pyramids, cubes, and higher order three-dimensional shapes; immobilized, conjugated, labeled and insolubilized nucleic acids, including nucleic acids hybridized or specifically bound to other soluble, insoluble, immobilized, conjugated or labeled nucleic acids; nucleic acid probes, targets and templates; sense, antisense and antigene nucleic acid strands; conjugated defined sequence segments and conjugated oligonucleotides, including oligonucleotides that are internally conjugated to provide closed-loop, single-ended or double-ended loop structures; branched, branched-chain, branched-comb, multi-chain and "Christmas tree" oligonucleotides; nucleic acid dendrons, dendrimers and nucleic acid conjugates formed by coulombic, affinity-based or covalent interactions with dendrons, dendrimers and other branched and hyperbranched structures; nucleotides comprising or capable of forming single-stranded, double-stranded, partially single-stranded, partially double-stranded, heteroduplex, triplex, quadruplex, chimeric and hybrid structures comprising natural or synthetic RNA, DNA or oligonucleotides comprising nucleotide analogs, derivatized nucleotides, nucleosides, nucleoside phosphates or backbone modifications. Selected nucleic acid **sequences hybridized to bifunctional synthetic** heteropolymers do not include unconjugated primers that hybridize to fixed primer-annealing sequences of aptamers selected from mixtures of random-sequence nucleic acids.

Summary of Invention Paragraph - BSTX:

[0375] The synthetic heteropolymers of the instant invention are not derived, selected or copied from wild-type biological nucleic acid molecules, sequences or groups of contiguous sequences, nor are they derived, isolated, selected or copied from heretofore-known **mutants**, genetic variants or nucleic acid molecules or sequences therefrom. At least one defined **sequence segment of each synthetic** heteropolymer or multivalent heteropolymeric hybrid structure of the instant invention is not only capable of specifically binding a nonoligonucleotide molecule, but is also synthetic. When used to describe a defined **sequence segment, synthetic means nonnaturally occurring, i.e., the defined sequence** segment is not heretofore known to occur in nature (sans human biotechnologic intervention) and is not heretofore known to be a biological recognition site. Biological recognition site means a first biological molecule or nucleic acid sequence that is heretofore known to specifically bind or recognize a second biological molecule or nucleic acid sequence. Unless otherwise specified, artificial and synthetic refer to willful products of human technology. Native, in nature, natural, naturally occurring, biological and organism, by contrast, refer to spontaneously occurring substances or beings that are not willful products of human-directed recombinant or transgenic technologies. In the case of hybrid plants and animals that have been identified and/or perpetuated by cross-breeding, selective breeding, cross-pollination, stem or limb grafting and the like, native, in nature, natural, naturally occurring, biological and organism mean only heretofore-known strains. Where the distinction between natural and synthetic is ambiguous, a heretofore-known substance, being or strain shall be considered natural for purposes of this disclosure, and a heretofore-unknown substance,

being or strain shall be considered synthetic.

Summary of Invention Paragraph - BSTX:

[0460] Selected nucleic acid **sequences include, but are not limited to, defined sequence segments of synthetic** heteropolymers, molecular machines, oligonucleotides, and RNA, DNA or denatured DNA sequences, including wild-type, **mutant** and recombinant biological nucleic acid **sequences; biological, engineered and synthetic** nucleic acid ligands, nucleic acid receptors, nucleic acid antibodies and nucleic acid sequences capable of participating in specific binding, catalytic and enzymatic reactions, e.g., aptamers, catalytic DNA and ribozymes; genomic, plasmid, cellular and transcribed or complementary nucleic acids, including DNA, cDNA and RNA; natural and **synthetic coding, noncoding, initiation, termination, promoter and regulatory sequences, including natural, synthetic, native or nonnative biological recognition sequences and therapeutic targets; natural and synthetic** oligonucleotides with defined topology, secondary or tertiary structure or three-dimensional shape, including rolling and circular nucleic acids, nucleic acid loops, stems, bulges, knots, pseudoknots, polygons, spheres, pyramids, cubes, and higher order three-dimensional shapes; immobilized, conjugated, labeled and insolubilized nucleic acids, including nucleic acids hybridized or specifically bound to other soluble, insoluble, immobilized, conjugated or labeled nucleic acids; nucleic acid probes, targets and templates; sense, antisense and antigene nucleic acid strands; conjugated defined sequence segments and conjugated oligonucleotides, including oligonucleotides that are internally conjugated to provide closed-loop or single-ended or double-ended loop structures; branched, branched-chain, branched-comb, multi-chain and Christmas tree oligonucleotides; nucleic acid dendrons, dendrimers and nucleic acid conjugates formed by coulombic, affinity-based or covalent interactions with dendrons, dendrimers and other branched and hyperbranched structures; single-stranded, double-stranded, partially single-stranded, partially double-stranded, heteroduplex, triplex, quadruplex, chimeric and hybrid structures comprising natural or synthetic RNA, DNA or oligonucleotides comprising nucleotide analogs, derivatized nucleotides or nucleoside triphosphates or backbone modifications. A defined sequence segment comprising a first molecular machine may hybridize or specifically bind to a selected nucleic acid sequence or selected molecule comprising a second molecular machine, thereby attaching the two molecular machines. The resulting product, which may be referred to as a single molecular machine or a pair of molecular machines, may attach to other molecular machines by methods described herein, including specific binding, hybridization, site-directed covalent attachment, pseudoirreversible attachment and the like.

Summary of Invention Paragraph - BSTX:

[0606] The proximity of the selected defined **sequence segments to one another within the synthetic** heteropolymer or multivalent heteropolymeric hybrid structure, which is controlled by the length, composition and three-dimensional structure of the spacer nucleotide and linker oligonucleotide sequences, is such that the binding of a molecule at one defined sequence segment can

modulate the affinity of another defined sequence segment for a second nonoligonucleotide molecule. Modulating the affinity refers to any increase or decrease in the association or dissociation rate constants that characterize the binding between a defined sequence segment and its specific binding partner. The binding of a molecule at one defined sequence segment can also modulate the activity of a molecule bound to another defined sequence segment. Modulating the activity refers to restoration, transduction or elimination in part or in full of the biological, chemical, optical, catalytic, mechanical, electrical or electrochemical activity of a selected molecule or nucleic acid sequence. For example, in a diagnostic assay, specific binding of a nonoligonucleotide molecule such as a receptor or ligand to a second defined **sequence segment of a synthetic heteropolymer may decrease the binding affinity of a first defined sequence** segment for a bound, inactive or partially inactive molecular effector. This results in displacement of the molecular effector and restoration of its activity. Thus, the presence of the selected receptor or ligand may be monitored by measuring activity of the molecular effector. In the case of a selected nucleic acid sequence, activity refers either to catalytic properties (e.g., ribozyme or catalytic DNA activity) or to information content (e.g., coding or **regulatory** properties). Modulation includes effects on catalytic activity, replication, transcription, translation and enzyme-dependent processes such as strand extension, ligation, amplification and the like.

Summary of Invention Paragraph - BSTX:

[0608] By positioning molecules so that binding or activity at a first defined sequence segment modulates binding or activity at a second defined **sequence segment, synthetic** heteropolymers of the present invention can be used to functionally couple a first selected molecule or nucleic acid sequence to a second selected molecule or nucleic acid sequence. For example, a first signal-generating molecule such as a fluorophore can be functionally coupled to a second signal-generating molecule such as a second fluorophore (e.g., a donor or acceptor), a light-driven or bioluminescent enzyme (e.g., an ATPase or **luciferase**) or an artificial reaction center (i.e., a molecule capable of photoinduced charge separation).

Summary of Invention Paragraph - BSTX:

[0611] Heteropolymeric functional coupling of the instant invention does not include the interaction between a ribozyme and its biological recognition site, i.e., the catalytic activity resulting from ribozyme-based recognition and cleavage of a biological nucleic acid sequence. Also outside the scope of the instant invention are ribozymes comprising **synthetic defined sequence** segments that bring the ribozyme catalytic element under allosteric control, i.e., by specific recognition of a selected molecule or selected nucleic acid sequence that **regulates** ribozyme catalytic activity.

Detail Description Paragraph - DETX:

[0756] For most applications, preferred methods for producing synthetic

heteropolymers include automated synthesis and biological methods, e.g., using recombinant DNA procedures. However, in some cases it is advantageous to simulate the function or evaluate the potential utility of a **synthetic heteropolymer using two or more defined sequence** segments that are either readily available or can be conveniently modified for a particular molecular assembly task. In such instances, it may be preferable to prepare a synthetic heteropolymer by less than ideal methods, e.g., by conjugating two defined sequence segments using covalent or pseudoirreversible means. Also, **synthetic heteropolymers comprising defined sequence** segments joined by nonnucleotidic linkages and/or linkers (e.g., nonnucleotide spacer groups, molecules or polymers) have utility in screening and analytical applications, e.g., to identify compounds or fractions having a desired catalytic activity and/or selectivity. For example, a population, generation or library of enzymes created by site-directed **mutagenesis** or directed in vitro evolution (e.g., random **mutagenesis** plus recombination) can be screened for activity in cleaving a bond connecting two defined sequence segments to which functionally coupled effectors are attached.

PGPUB-DOCUMENT-NUMBER: 20020028444

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020028444 A1

TITLE: METHOD AND KITS FOR PREPARING MULTICOMPONENT NUCLEIC ACID
CONSTRUCTS

PUBLICATION-DATE: March 7, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|------------------|-------------|-------|---------|---------|
| HARNEY, PETER D. | ALISO VIEJO | CA | US | |
| HARNEY, JENNIFER | ALISO VIEJO | CA | US | |

APPL-NO: 09/ 220398

DATE FILED: December 24, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued
prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09220398 A1 19981224 parent continuation-in-part-of 08877034 19970616 US
GRANTED parent-patent 6277632 US child 09220398 A1 19981224 parent
a-371-of-international PCT/US97/10523 19970616 WO UNKNOWN
non-provisional-of-provisional 60019869 19960617 US

US-CL-CURRENT: 435/6

ABSTRACT:

The invention provides a highly efficient, rapid, and cost effective method of linking nucleic acid components in a predetermined order to produce a nucleic acid multicomponent construct. The invention further provides nucleic acid components, each nucleic acid component comprising a double stranded nucleic acid molecule having at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to either a terminal sequence in a separate nucleic acid component or to a sequence in a linking nucleic acid molecule so as to allow for specific annealing of complementary sequences and linkage of the components in a predetermined order. Kits containing reagents required to practice the method of the invention are also provided.

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. Ser. No.
08/877,034, filed Jun. 16, 1997, which claims the benefit of a previously

filed Provisional Application No. 60/019,869 filed Jun. 17, 1996, the specifications of which are hereby incorporated by reference.

----- KWIC -----

Detail Description Paragraph - DETX:

[0139] As used herein, the terms "exon" and "exonic sequence" denotes nucleic acid sequences, or exon "modules", that can, for instance, encode portions of proteins or polypeptide chains, such as corresponding to naturally occurring exon sequences or naturally occurring exon sequences which have been mutated (e.g. point mutations, truncations, fusions), as well as nucleic acid sequences from "synthetic exons" including sequences of purely random construction. However, the term "exon", as used in the present invention, is not limited to protein-encoding sequences, and may comprises nucleic acid sequences of other function, including nucleic acids of "intronic origin" which give rise to, for example, ribozymes or other nucleic acid structure having some defined chemical function.

Detail Description Paragraph - DETX:

[0156] As suggested above, the inducible promoters of the present invention include those which are not naturally occurring promoters but rather synthetically derived inducible promoter systems which may make use of prokaryotic transcriptional repressor proteins. The advantage of using prokaryotic repressor proteins in the invention is their specificity to a corresponding bacterial operator binding site, which can be incorporated into the synthetic inducible promoter system. These prokaryotic repressor proteins have no natural eukaryotic gene targets and affect only the effector of suppression gene which is put under the transcriptional control of the inducible synthetic promoter. This system thereby avoids undesirable side-effects resulting from unintentional alteration of the expression of nontargeted eucaryotic genes when the inducible promoter is induced. A preferred example of this type of inducible promoter system is the tetracycline-regulated inducible promoter system. Various useful versions of this promoter system have been described (see Shockett and Schatz (1996) Proc. Natl. Acad. Sci. USA 93: 5173-76 for review). As suggested above, these tetracycline-regulated systems generally make use of a strong eucaryotic promoter, such as human cytomegalovirus (CMV) immediate early (IE) promoter/enhancer and a tet resistance operator (tetO) which is bound by the tet repressor protein. In a preferred embodiment, the system involves a modified version of the tet repressor protein called a reverse transactivator (rtTA, or rtTA-nls, which contains a nuclear localization signal) which binds tetO sequences only in the presence of the tet derivatives doxycycline or anhydrotetracycline. Using this system, a synthetic human CMV/IE-tetO-promoter driven construct could be induced by 3 orders of magnitude in 20 hrs by the addition of the tet derivatives (see Gossen et al. (1995) Science 268: 1766-9). Thus this system can be used to make the effector of suppression genes of the present invention inducible in response to the delivery of tetracycline derivatives to the targeted eucaryotic cell. Alternatively, a tet repressor

fused to a transcriptional activation domain of VP16 (tTA) can be used to drive expression of the inducible promoter of the present invention. In this instance, transcriptional activation of a synthetic human CMV/IE- tetO-promoter driven construct is achieved by the removal of tetracycline since the tTA activator only binds to the tetO in the absence of tet (see Gossen and Bujard (1992) Proc. Natl. Acad. Sci. USA 89: 5547-51). Other synthetic inducible promoter systems are also available for use in the present invention. For example, a lac repressor-VP16 fusion which exhibits a "reverse" DNA binding phenotype (i.e., analogous to rtTA described above, it only binds the lacO operator sequence in the presence of the inducer IPTG) (see Lambowitz and Belfort (1993) Annu Rev Biochem 62: 587-622). This particular synthetic inducible promoter is approximately 1000-fold inducible in the presence of IPTG. Since neither the tet repressor gene nor the lac repressor gene occurs naturally in a eukaryotic cell, systems involving synthetic inducible promoter constructs such as these rely on the further delivery of an expressible copy of the appropriate prokaryotic repressor gene. Suitable expression cassettes for this purpose are readily available for heterologous expression in many different eukaryotic cells including various yeast species and mammalian cells.

Detail Description Paragraph - DETX:

[0158] Yet another vector element of the present invention is fusion polypeptide-encoding element which can be fused to the coding region of any insert gene of interest. For example, in certain applications it is useful to be able to mark a particular gene product with a tag so that the localization and function of the gene product can be easily monitored. A particularly preferred version of a molecular tag fusion polypeptide element is the green fluorescent protein or GFP (see e.g. Misteli and Spector (1997) Nature Biotechnology 15: 961-4; and Gerdes and Kaether (1996) FEBS Lett 389: 44-7 for review). This fusion tag polypeptide emits green (approximately 510 nm wavelength) light upon excitation by a particular wavelength of incident light (approximately 400 to 480 nm, depending upon the form of GFP). Various versions of GFP coding sequences, including those whose codon usage has been humanized and those whose emission spectra have been "red-shifted," are commercially available and can be readily adapted to GEOS methodology. Applications of GFP include in situ localization of a linked gene of interest, as well as facile monitoring of expression and tropism in various cell mediated expression studies.

Detail Description Paragraph - DETX:

[0169] A vector may be assembled from multiple individual nucleic acid components, including, without limitation, nucleic acid components which incorporate one or more of the following: (a) origin of replication (bacterial, viral, phage, yeast, mammalian, eukaryotic), (b) selectable markers (antibiotic resistance, drug resistance, mutagenic resistance), (c) promoters (phage, bacterial, yeast, eukaryotic, mammalian), (d) regulatory elements or genes (repressors, enhances), (e) structural genes, (f) fragments of structural genes, (g) translational elements (Shine-Delgarno element, Kozak sequence), (h) terminators of transcription, (i) regulators of mRNA stability (degradation

signals, translational **regulators**), (j) protein encoded elements specifying cellular location (leader sequence, KDEL, CAAX box, nuclear targeting elements), (k) recombination elements (Lox-CRE, M13 ori), (l) **mutagenized genes**, (m) **protein domain encoded regions**, (n) **synthetic** multiple cloning sites, (o) unique restriction enzyme or DNA cleavage sites, (p) site for covalent or non covalent attachment of a biological or chemical molecule (see "Handle").

Claims Text - CLTX:

15. The method of claim 1, wherein the nucleic acid component encodes a biological functionality selected from the group consisting of origin of replication, selectable marker, transcriptional **regulatory** element, structural gene or fragment thereof, transcription termination signal, translational **regulatory** sequence, **regulators** of mRNA stability, cellular localization signal, recombination elements, **mutagenized genes**, **protein domain encoded regions**, **synthetic** multiple cloning sites, unique restriction enzyme or DNA cleavage sites, and site for covalent or non covalent attachment of a biological or chemical molecule."

PGPUB-DOCUMENT-NUMBER: 20020015979

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020015979 A1

TITLE: VEHICLES FOR STABLE TRANSFER OF GREEN FLUORESCENT PROTEIN GENE
AND
METHODS OF USE FOR SAME

PUBLICATION-DATE: February 7, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|-----------------|-------|---------|---------|
| LINK,, CHARLES J. JR. | CLIVE | IA | US | |
| LEVY, JOHN P. | WEST DES MOINES | IA | US | |
| WANG, SUMING | DES MOINES | IA | US | |
| SEREGINA, TATIANA | WEST DES MOINES | IA | US | |

APPL-NO: 08/ 786531

DATE FILED: January 21, 1997

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60010371 19960122 US

US-CL-CURRENT: 435/69.1,435/189 ,435/252.3 ,435/320.1 ,435/69.7 ,435/70.3 ,435/70.4 ,536/23.4 ,536/23.5 ,536/24.1 ,536/24.2

ABSTRACT:

The present invention describes an efficient retroviral or viral based method that allows easy and quick identification of gene transfer in living, transduced mammalian cells. Retroviral and viral vector producer cells were generated containing a **gene for an improved humanized** red-shifted, Green Fluorescent Protein (hRGFP) which increases the resulting fluorescence yield after excitation. This **humanized, red-shifted GFP (hRGFP) gene** was cloned into several vectors and transfected into various packaging cell lines to produce vibrant green fluorescence after excitation with blue light at 450-490 nm. These vectors represent a substantial advance over currently available gene transfer marking systems or wild-type **GFP** marker systems none of which have been stably transfected into cells.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part of provisional application

----- KWIC -----

Abstract Paragraph - ABTX:

The present invention describes an efficient retroviral or viral based method that allows easy and quick identification of gene transfer in living, transduced mammalian cells. Retroviral and viral vector producer cells were generated containing a gene for an improved humanized red-shifted, Green Fluorescent Protein (hRGFP) which increases the resulting fluorescence yield after excitation. This humanized, red-shifted GFP (hRGFP) gene was cloned into several vectors and transfected into various packaging cell lines to produce vibrant green fluorescence after excitation with blue light at 450-490 nm. These vectors represent a substantial advance over currently available gene transfer marking systems or wild-type GFP marker systems none of which have been stably transfected into cells.

Summary of Invention Paragraph - BSTX:

[0009] A novel marker gene is now available that will alleviate these cumbersome and time consuming steps for detecting gene transfer. The Green Fluorescent Protein (GFP) is a vibrant green bioluminescent marker which offers outstanding properties, the gene has been sequenced, humanized and is commercially available through several sources, however there has been much difficulty in finding a suitable transformation vehicle that will give stable expression in mammalian cells.

Summary of Invention Paragraph - BSTX:

[0015] The present invention describes the cloning and characterization of amphotropic retroviral vectors capable of demonstrating efficient, stable transfer of humanized, red shifted GFP (hRGFP) gene into mammalian cells. Living cells transfected and/or transduced with hRGFP have a stable, bright green fluorescence after excitation with blue light.

Brief Description of Drawings Paragraph - DRTX:

[0018] FIG. 2 shows the retroviral constructs containing the red shift, humanized Green Fluorescent Protein. The wild-type GFP and the humanized, red shifted GFP gene were cloned into the pLNCX retroviral backbone. Plasmid pLNCG was constructed by PCR amplification of a wild-type GFP containing DNA fragment and subsequent subcloning into pLNCX. LTR, long terminal repeat; pA, polyadenylation signal; arrows indicate transcriptional start sites; .PSI..sup.+ indicates the presence of the viral packaging sequence; GFP, wild-type green fluorescent protein; hRGFP, humanized, red shifted GFP.

Brief Description of Drawings Paragraph - DRTX:

[0032] FIGS. 7A-7G are the DNA sequence of the pHGFP-S65T humanized GFP vector including restriction sites.

Detail Description Paragraph - DETX:

[0050] As used herein, "nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. Generally, DNA segments encoding the proteins provided by this invention are assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

Detail Description Paragraph - DETX:

[0075] The key to expression seems to lie in the 3 amino acid residues which form a cyclized chromophore. Specifically, the serine at position 65 is a site at which several amino acid replacements shows increased intensity and quickens the rate of chromophore formation. The red shift may be responsible for advancing expression to this level. However, humanizing of the coding sequences seems to be providing the dominant contribution. Jellyfish are extremely divergent from mammals and consequently have different codon usage. This may present a translation challenge for mammalian cells of enough proportion to prevent a build up of detectable GFP. The HGS65T GFP gene contains 169 codon changes (Clonetech) representing 71% of the gene. The above results show that this is a superior marker gene to follow retroviral and viral transduction.

Detail Description Paragraph - DETX:

[0077] In vivo retroviral transduction experiments may also take advantage of this marker system. PA317-HGS65T VPC cells injected into an established subcutaneous tumor cell will transduce the dividing tumor cells. VPC's used in black mice systems will be destroyed by the immune system within 2 weeks after injection. The F.A.C.S. sorting capabilities also opens up some doors of opportunity for leukemia trials. Overall, this evidence demonstrated that humanized red shift GFP has the potential of becoming a major player in gene therapy.

Detail Description Paragraph - DETX:

[0078] The instant invention demonstrates the effectiveness of a humanized, red shifted mutant GFP by retroviral and viral mediated gene transfer into human tumor cells and murine fibroblasts. A few molecular genetics groups have now reported mutations of the wild-type GFP gene which can generate GFP gene products with modified excitation and emission spectra. See for e.g. Heim, R.,

et al. (1994) Proc Natl Acad Sci, USA 91:12501-12504. The longer wavelength excitation peak (475 nm) of native A. Victoria **GFP** has lower amplitude than its shorter wavelength excitation peak (470-490 nm) with fluorescence amplitudes from 4-6 fold greater than from the wild-type gene product. Heim, R. (1995) Nature 373:663-664. Interestingly, this **mutant** also had more rapid formation of the fluorochrome. Id. Furthermore, the **mutate**, red shifted **GFP** had its codons modified to usage common in mammals (Dr. Sergei Zolotukhin and Dr. Nicholas Muzyczka, University of Florida, unpublished results). The inventors have evaluated this humanized version of a serine-65 to threonine codon **mutant** that demonstrates excitation at 490 nm and emissions at 510 nm in current gene transfer experiments. Comparisons between the wild-type **GFP** and the humanized, serine-65 red shifted **mutant** (hRGFP) demonstrated substantial improvement in fluorescence expression after either transfection and retroviral mediated **GFP** gene transfer (Table 1).

Detail Description Paragraph - DETX:

[0099] These results demonstrate the effectiveness of a **humanized, red-shifted mutant GFP by retroviral mediated gene** transfer into human tumor cells and murine fibroblasts.

Detail Description Table CWU - DETL:

2TABLE 2 SEQUENCE AND RESTRICTION SITE INFORMATION (SEQ ID NO. 1)
 phGFP-S65T Humanized **GFP** Vector GenBank Accession #: U43284 Location of features Human cytomegalovirus (CMV) immediate early promoter: 152-739 Enhancer region: 229-635 TATA box: 724-730 T7 promoter: 784-803 Green fluorescent protein gene (S65T variant) Start codon (ATG): 826-828; Stop codon: 1543-1545 **GFP** fluorescent chromophore: 1021-1029 SV40 small t antigen intron: 1642-1706 SV40 early mRNA polyadenylation signal Polyadenylation signals: 2312-2317 & 2341-2346 mRNA 3' ends: 2350 & 2362 SV40 origin of replication: 2805-2740 pBR322 plasmid replication origin: 2767-3347 M13 single-strand DNA origin: 3367-3934 **Synthetic supF gene**: 4145-3947 Geneology From To 1549 811 pCDM7 vector backbone (Not I-Hind III) 1 151 Fragment from the Rous Sarcoma Virus (RSV) LTR 152 738 Fragment from Human Cytomegalovirus (CMV) containing the immediate early promoter 812 1548 **Synthetic GFP gene** using optimal human codons 817 829 Synthetic Kozak consensus translation initiation sequence 829 831 Additional valine not present in wt **GFP** 1021 1023 865T **mutation in GFP** chromophore replacing serine 65 with threonine [Heim, R. et al. (1995) Nature 373:663-664] 1565 2174 Fragment from SV40 providing small antigen intron 2175 2415 Fragment from SV40 providing polyadenylation signals 2416 2759 Fragment from SV40 providing origin of replication 2767 3347 Fragment from pBR322 providing origin of replication 3367 3934 Fragment from M13 providing single-stranded DNA origin 3947 4145 **Synthetic supF gene** Propagation in E. coli Suitable host strain: MC1061/P3 Selectable Marker: The supF gene confers resistance to ampicillin (25-40 .mu.g/ml) and tetracycline (7.5-10 .mu.g/ml) to MC1061/P3 due to expression of a tRNA that suppresses amber **mutations** in the ampicillin and tetracycline genes on the P3 plasmid. E. coli replication origin: pBR322 (rop.sup.-) Copy number: = 100-200 Plasmid incompatibility group: pMB1/ColE1

US-PAT-NO: 6495318

DOCUMENT-IDENTIFIER: US 6495318 B2

TITLE: Method and kits for preparing multicomponent nucleic acid constructs

DATE-ISSUED: December 17, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------|---------------------|-------|----------|---------|
| Harney, Peter D. | late of Aliso Viejo | CA | N/A | N/A |

APPL-NO: 09/ 220398

DATE FILED: December 24, 1998

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of U.S. Ser. No. 08/877,034, filed Jun. 16, 1997, now U.S. Pat. No. 6,277,632, which claims the benefit of a previously filed Provisional Application No. 60/019,869 filed Jun. 17, 1996, which is a 371 of PCT/US97/10523, filed Jun. 16, 1997, the specifications of which are hereby incorporated by reference.

US-CL-CURRENT: 435/6; 435/254.2 ; 435/320.1 ; 435/91.1 ; 435/91.2 ; 536/22.1 ; 536/24.3 ; 536/24.33 ; 536/24.5

ABSTRACT:

The invention provides a highly efficient, rapid, and cost effective method of linking nucleic acid components in a predetermined order to produce a nucleic acid multicomponent construct. The invention further provides nucleic acid components, each nucleic acid component comprising a double stranded nucleic acid molecule having at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to either a terminal sequence in a separate nucleic acid component or to a sequence in a linking nucleic acid molecule so as to allow for specific annealing of complementary sequences and linkage of the components in a predetermined order. Kits containing reagents required to practice the method of the invention are also provided.

43 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Detailed Description Text - DETX:

As used herein, the terms "exon" and "exonic sequence" denotes nucleic acid sequences, or exon "modules", that can, for instance, encode portions of proteins or polypeptide chains, such as corresponding to naturally occurring exon sequences or naturally occurring exon sequences which have been mutated (e.g. point mutations, truncations, fusions), as well as nucleic acid sequences from "synthetic exons" including sequences of purely random construction. However, the term "exon", as used in the present invention, is not limited to protein-encoding sequences, and may comprises nucleic acid sequences of other function, including nucleic acids of "intronic origin" which give rise to, for example, ribozymes or other nucleic acid structure having some defined chemical function.

Detailed Description Text - DETX:

As suggested above, the inducible promoters of the present invention include those which are not naturally occurring promoters but rather synthetically derived inducible promoter systems which may make use of prokaryotic transcriptional repressor proteins. The advantage of using prokaryotic repressor proteins in the invention is their specificity to a corresponding bacterial operator binding site, which can be incorporated into the synthetic inducible promoter system. These prokaryotic repressor proteins have no natural eukaryotic gene targets and affect only the effector of suppression gene which is put under the transcriptional control of the inducible synthetic promoter. This system thereby avoids undesirable side-effects resulting from unintentional alteration of the expression of nontargeted eucaryotic genes when the inducible promoter is induced. A preferred example of this type of inducible promoter system is the tetracycline-regulated inducible promoter system. Various useful versions of this promoter system have been described (see Shockett and Schatz (1996) Proc. Natl. Acad. Sci. USA 93: 5173-76 for review). As suggested above, these tetracycline-regulated systems generally make use of a strong eucaryotic promoter, such as human cytomegalovirus (CMV) immediate early (IE) promoter/enhancer and a tet resistance operator (tetO) which is bound by the tet repressor protein. In a preferred embodiment, the system involves a modified version of the tet repressor protein called a reverse transactivator (rtTA, or rtTA-nls, which contains a nuclear localization signal) which binds tetO sequences only in the presence of the tet derivatives doxycycline or anhydrotetracycline. Using this system, a synthetic human CMV/IE-tetO-promoter driven construct could be induced by 3 orders of magnitude in 20 hrs by the addition of the tet derivatives (see Gossen et al. (1995) Science 268: 1766-9). Thus this system can be used to make the effector of suppression genes of the present invention inducible in response to the delivery of tetracycline derivatives to the targeted eucaryotic cell. Alternatively, a tet repressor fused to a transcriptional activation domain of VP16 (tTA) can be used to drive expression of the inducible promoter of the present invention. In this instance, transcriptional activation of a synthetic human CMV/IE- tetO-promoter driven construct is achieved by the removal of tetracycline since the tTA activator only binds to the tetO in the absence of tet (see Gossen and Bujard (1992) Proc. Natl. Acad. Sci. USA 89: 5547-51).

Other synthetic inducible promoter systems are also available for use in the present invention. For example, a lac repressor-VP16 fusion which exhibits a "reverse" DNA binding phenotype (i.e., analogous to rtTA described above, it only binds the lacO operator sequence in the presence of the inducer IPTG) (see Lambowitz and Belfort (1993) Annu Rev Biochem 62: 587-622). This particular synthetic inducible promoter is approximately 1000-fold inducible in the presence of IPTG. Since neither the tet repressor gene nor the lac repressor **gene occurs naturally in a eukaryotic cell, systems involving synthetic** inducible promoter constructs such as these rely on the further delivery of an expressible copy of the appropriate prokaryotic repressor gene. Suitable expression cassettes for this purpose are readily available for heterologous expression in many different eukaryotic cells including various yeast species and mammalian cells.

Detailed Description Text - DETX:

Yet another vector element of the present invention is fusion polypeptide-encoding element which can be fused to the coding region of any insert gene of interest. For example, in certain applications it is useful to be able to mark a particular gene product with a tag so that the localization and function of the gene product can be easily monitored. A particularly preferred version of a molecular tag fusion polypeptide element is the green fluorescent protein or **GFP** (see e.g. Misteli and Spector (1997) Nature Biotechnology 15: 961-4; and Gerdes and Kaether (1996) FEBS Lett 389: 44-7 for review). This fusion tag polypeptide emits green (approximately 510 nm wavelength) light upon excitation by a particular wavelength of incident light (approximately 400 to 480 nm, depending upon the form of **GFP**). Various versions of **GFP** coding **sequences, including those whose codon usage has been humanized** and those whose emission spectra have been "red-shifted," are commercially available and can be readily adapted to GEOS methodology. Applications of **GFP** include in situ localization of a linked gene of interest, as well as facile monitoring of expression and tropism in various cell mediated expression studies.

Detailed Description Text - DETX:

A vector may be assembled from multiple individual nucleic acid components, including, without limitation, nucleic acid components which incorporate one or more of the following: (a) origin of replication (bacterial, viral, phage, yeast, mammalian, eukaryotic), (b) selectable markers (antibiotic resistance, drug resistance, **mutagenic** resistance), (c) promoters (phage, bacterial, yeast, eukaryotic, mammalian), (d) **regulatory** elements or genes (repressors, enhancers), (e) structural genes, (f) fragments of structural genes, (g) translational elements (Shine-Delgarno element, Kozak sequence), (h) terminators of transcription, (i) **regulators** of mRNA stability (degradation signals, translational **regulators**), (j) protein encoded elements specifying cellular location (leader sequence, KDEL, CAAX box, nuclear targeting elements), (k) recombination elements (Lox-CRE, M13 ori), (l) **mutagenized genes, (m) protein domain encoded regions, (n) synthetic** multiple cloning sites, (o) unique restriction enzyme or DNA cleavage sites, (p) site for

covalent or non covalent attachment of a biological or chemical molecule (see "Handle").

Claims Text - CLTX:

15. The method of claim 1, wherein the nucleic acid component encodes a biological functionality selected from the group consisting of origin of replication, selectable marker, transcriptional **regulatory** element, structural gene or fragment thereof, transcription termination signal, translational **regulatory** sequence, **regulators** of mRNA stability, cellular localization signal, recombination elements, **mutagenized genes, protein domain encoded regions, synthetic** multiple cloning sites, unique restriction enzyme or DNA cleavage sites, and site for covalent or non covalent attachment of a biological or chemical molecule.

US-PAT-NO: 6489141

DOCUMENT-IDENTIFIER: US 6489141 B1

TITLE: Nucleic acid sequence and methods for selectively expressing a protein in a target cell or tissue

DATE-ISSUED: December 3, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|------------------|-------|----------|---------|
| Frazer; Ian Hector | St. Lucia | N/A | N/A | AU |
| Zhou; Jian | late of Jindalee | N/A | N/A | AU |

APPL-NO: 09/ 479645

DATE FILED: January 7, 2000

PARENT-CASE:

This application is a continuation-in-part application of co-pending International Patent Application No. PCT/AU98/00530 filed Jul. 9, 1998, which designates the United States, and which claims priority of Australian Patent Application Nos. PO7765 filed Jul. 9, 1997 and PO9467 filed Sep. 11, 1997.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|--------------------|
| AU | 7765 | July 9, 1997 |
| AU | 9467 | September 11, 1997 |
| AU | 8078 | January 8, 1999 |

US-CL-CURRENT: 435/69.1; 435/320.1 ; 435/325 ; 435/455 ; 435/91.4 ; 435/91.41 ; 435/91.42 ; 514/44

ABSTRACT:

A synthetic polynucleotide and a method are disclosed for selectively expressing a protein in a target cell or tissue of a mammal. Selective expression is effected by replacing at least one existing codon of a parent polynucleotide encoding a protein of interest with a synonymous codon to produce a synthetic polynucleotide having altered translational kinetics compared to the parent polynucleotide. The synonymous codon is selected such that it has a higher translational efficiency in the target cell or tissue relative to one or more other cells or tissues of the mammal.

60 Claims, 35 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

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Drawing Description Text - DRTX:

FIG. 6A, comprising FIGS. 6A-1, 6A-2, and 6A-3, is a series of images, each of which depicts a confocal micrograph showing expression of GFP in COS-1 cells transfected with wild-type gfp (wt) (FIG. 6A-2) or a synthetic gfp gene (FIG. 6A-3) carrying codons used at relatively high frequency by papillomavirus genes (p). FIG. 6A-1 shows results from a mock transfection.

Drawing Description Text - DRTX:

FIG. 7, comprising FIGS. 7A through 7L, is a series of images, each of which depicts the expression pattern of GFP in vivo from wild-type gfp gene, or a synthetic gfp gene carrying codons used at relatively high frequency by papillomavirus genes. Using a gene gun, mice were shot with PGFP (FIGS. 7A through 7F) and GFP (FIGS. 7G through 7L) expression plasmids encoding GFP protein. A transverse section of the mouse skin section shows where the gfp gene is expressed. Bright-field photographs (FIGS. 7A, 7B, 7G, and 7H) of the same section where dermis (D) epidermis (E) are highlighted are shown to identify the location of fluorescence in the epidermis. Arrows indicate fluorescent signals.

Detailed Description Text - DETX:

As the major limitation to expression of the wild type BPV L1 and L2 genes appeared to be translational in our system, we wished to test whether this limitation reflected a limited availability of the appropriate tRNA species for gene translation. As transient expression of the synthetic genes within intact cells can be regulated by many factors, we tested our hypothesis in a cell free system using rabbit reticulocyte lysate (RRL) or wheat germ lysate to examine gene translation. Similar amounts of plasmids expressing the wild type or synthetic humanized BPV1 L1 gene were added to a T7-DNA polymerase coupled RRL transcription/translation system in the presence of .sup.35 S-methionine. After 20 minutes, translated proteins were separated by SDS PAGE and visualized by autoradiography. Efficient translation of the modified L1 gene was observed (FIG. 4A, lane 2), while translation of the wild type BPV1 L1 sequence resulted in a weak 55 kDa L1 band (FIG. 4A, lane 1). We reasoned that although the wild type sequence was not optimized for translation in RRL, some translation would occur, as there would be no cellular mRNA species competing for the 'rare' codons present in the wild type L1 sequence. The above data suggest that the observed difference in efficiency of translation of the wild type and synthetic humanized L1 genes is a consequence of limited availability of the tRNAs required for translation of the rare codons present in the wild type gene. We therefore expected that addition of excess tRNA to the in vitro translation system would overcome the inhibition of translation of the wild type L1 gene. To address this question, 10.sup.-5 M aminoacyl-tRNAs from yeast were added

into the RRL translation system, and L1 protein synthesis was assessed. Introduction of exogenous tRNAs resulted in a dramatic improvement in translation of the wild type L1 sequence, which now gave a yield of L1 protein comparable to that observed with the synthetic humanized L1 sequence (SEQ ID NO:3) (FIG. 4A). Enhancement of translation of the wild type L1 gene (SEQ ID NO:1) by aminoacyl-tRNA was dose-dependent, with an optimum efficiency at 10. sup.-5 M tRNA. As addition of exogenous tRNA improved the yield of L1 protein translated from the wild type L1 gene sequence (SEQ ID NO:1), we assessed the speed of translation of wild type and humanized L1 mRNA. Samples were collected from the translation mixture every 2 minutes, starting at the 8th minute. Translation of L1 (SEQ ID NO:2, 4) from the wild type sequence (SEQ ID NO:1) was much slower than from the humanized L1 sequence (SEQ ID NO:3) (FIG. 4B), and the retardation of translation could be completely overcome by adding exogenous tRNA from commercially available yeast tRNA. Yeast tRNA was chosen in the above analysis because the codon usage in yeast is similar to that of papillomavirus (Table 1). Addition of exogenous tRNA did not significantly improve the translation of the humanized L1 gene (SEQ ID NO:3), indicating that this sequence was optimized with regard to codon usage for the rabbit reticulocyte translation machinery (FIG. 4B). In separate experiments we established that wt L1 translation could also be enhanced by liver tRNA (FIG. 4), and by tRNAs extracted from bovine skin epidermis, which presumably constitutes a mixture of tRNAs from differentiated and undifferentiated cells.

Detailed Description Text - DETX:

To construct a modified gfp gene (SEQ ID NO:11) using papillomavirus preferred codons (PGFP), 6 pairs of oligonucleotides were synthesized. Each pair of oligonucleotides has restriction sites incorporated and was used to amplify gfp using a humanized gfp gene (SEQ ID NO:9) (GIBCO) as template. The PCR fragments were ligated into the pUC18 vector to produce pUCPGFP. The PGFP gene was sequenced, and cloned into BamHI site of the same mammalian expression vector, pCDNA3, under the CMV promoter. The DNA and deduced amino acid sequences of the humanized gfp gene are shown in FIGS. 1C. Mutations introduced into the wild type gfp gene (SEQ ID NO:9) to produce the Pgfp gene (SEQ ID NO:11) are indicated above the corresponding nucleotide residues of the wild-type sequence.

Detailed Description Text - DETX:

To further confirm that condon usage can alter gene expression in mammalian cells, we made a further variant on a synthetic gfp gene modified for optimal expression in eukaryotic cells (Zolotukhin, et al., 1996. J. Virol. 70:4646-4654). In our variant, codons optimized for expression in eukaryotic cells were substituted by those preferentially used in papillomavirus late genes. Of 240 codons in the humanized gfp gene (SEQ ID NO:9), which expresses high levels of fluorescent protein in cultured cells, 156 were changed to the corresponding papillomavirus late gene-preferred codons to produce a new gfp gene (SEQ ID NO:11) designated Pgfp. Expression of Pgfp (SEQ ID NO:11) in undifferentiated cells was compared with that of humanized gfp (SEQ ID NO:9). COS-1 cells transfected with the humanized gfp (SEQ ID NO:9) produced a bright

fluorescent signal after 24 hrs, while cells transfected with Pgfp (SEQ ID NO:11) produced only a faint fluorescent signal (FIG. 6A-3). To confirm that this difference reflected differing translational efficacy, gfp specific mRNA was tested in both transfections and found not to be significantly different (FIG. 6B). Thus, codon usage and corresponding tRNA availability apparently determines the observed restriction of expression of PV late genes, and modification of codon usage in other genes similarly prevents their expression in undifferentiated cells.

Detailed Description Text - DETX:

Synthetic gfp genes were constructed in which a single artificial start codon (ATG) followed by a stretch of five identical codons is fused in frame immediately upstream of a gfp coding sequence. A reverse oligonucleotide primer (SEQ ID NO:219; sequence complementary to the termination codon for GFP, is underlined), and a suite of forward oligonucleotide primers (SEQ ID NO: 160 through 218; the first codon of GFP, is underlined) were synthesized and used for PCR amplification of a humanized gfp gene (SEQ ID NO:158) (GIBCO) as template with Taq DNA polymerase (Amplification parameters: 95.degree. C./30 sec; 52.degree. C./30 sec; 72.degree. C./1 min; 30 cycles). The amplified fragments have nucleic acid sequences and deduced amino acid sequences as shown in SEQ ID NO:35 through 157.

Detailed Description Paragraph Table - DETL:

Asp Thr Leu Thr Thr Arg Ser Gly Thr Glu Val Gly Pro Gln Leu His 305 310 315
 320 Val Arg Tyr Ser Leu Ser Thr Ile His Glu Asp Val Glu Ala Ile Pro 325 330
 335 Tyr Thr Val Asp Glu Asn Thr Gln Gly Leu Ala Phe Val Pro Leu His 340 345
 350 Glu Glu Gln Ala Gly Phe Glu Glu Ile Glu Leu Asp Asp Phe Ser Glu 355 360
 365 Thr His Arg Leu Leu Pro Gln Asn Thr Ser Ser Thr Pro Val Gly Ser 370 375
 380 Gly Val Arg Arg Ser Leu Ile Pro Thr Arg Glu Phe Ser Ala Thr Arg 385 390
 395 400 Pro Thr Gly Val Val Thr Tyr Gly Ser Pro Asp Thr Tyr Ser Ala Ser 405
 410 415 Pro Val Thr Asp Pro Asp Ser Thr Ser Pro Ser Leu Val Ile Asp Asp 420
 425 430 Thr Thr Thr Thr Pro Ile Ile Ile Ile Asp Gly His Thr Val Asp Leu 435
 440 445 Tyr Ser Ser Asn Tyr Thr Leu His Pro Ser Leu Leu Arg Lys Arg Lys 450
 455 460 Lys Arg Lys His Ala 465 <200> SEQUENCE CHARACTERISTICS:
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 <213> ORGANISM: Artificial Sequence <220> FEATURE: <221>
 NAME/KEY: CDS <222> LOCATION: (1)..(1410) <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence Bovine
 papillomavirus type 1 L2 open reading frame (humanized) <400> SEQUENCE:
 7 atg agc gcc cgc aag aga gtg aag cgc gcc agc gcc tac gac ctg tac 48 Met Ser
 Ala Arg Lys Arg Val Lys Arg Ala Ser Ala Tyr Asp Leu Tyr 1 5 10 15 agg acc tgc
 aag cag gcc ggc aca tgt cca cca gat gtg atc cga aag 96 Arg Thr Cys Lys Gln Ala
 Gly Thr Cys Pro Pro Asp Val Ile Arg Lys 20 25 30 gtg gag ggc gac acc atc gcc
 gac aag atc ctg aag ttc ggc ggc ctg 144 Val Glu Gly Asp Thr Ile Ala Asp Lys
 Ile Leu Lys Phe Gly Gly Leu 35 40 45 gcc atc tac ctg ggc ggc ctg ggc atc gga
 aca tgg tct acc ggc agg 192 Ala Ile Tyr Leu Gly Gly Leu Gly Ile Gly Thr Trp
 Ser Thr Gly Arg 50 55 60 gtg gcc gcc ggc ggc tca cca agg tac acc cca ctg cgc
 acc gcc ggc 240 Val Ala Ala Gly Gly Ser Pro Arg Tyr Thr Pro Leu Arg Thr Ala
 Gly 65 70 75 80 tcc acc tcc tcc ctg gcc tcc atc gga tcc aga gcc gtg acc gcc

ggg 288 Ser Thr Ser Ser Leu Ala Ser Ile Gly Ser Arg Ala Val Thr Ala Gly 85 90
 95 acc cgc ccc tcc atc ggc gcg ggc atc cct ctg gac acc ctg gaa act 336 Thr
 Arg Pro Ser Ile Gly Ala Gly Ile Pro Leu Asp Thr Leu Glu Thr 100 105 110 ctt
 ggg gcc ctg cgc cct ggc gtg tac gag gac acc gtg ctg ccc gaa 384 Leu Gly Ala
 Leu Arg Pro Gly Val Tyr Glu Asp Thr Val Leu Pro Glu 115 120 125 gcc cct gcc
 atc gtg acc cct gac gcc gtg cct gca gac tcc ggc ctg 432 Ala Pro Ala Ile Val
 Thr Pro Asp Ala Val Pro Ala Asp Ser Gly Leu 130 135 140 gac gcc ctg tcc atc
 ggc aca gac tcc tcc acc gag acc ctg atc acc 480 Asp Ala Leu Ser Ile Gly Thr
 Asp Ser Ser Thr Glu Thr Leu Ile Thr 145 150 155 160 ctg ctg gag cct gag ggc
 ccc gaa gac ata gcc gtg ctg gaa ctc cag 528 Leu Leu Glu Pro Glu Gly Pro Glu
 Asp Ile Ala Val Leu Glu Leu Gln 165 170 175 ccc ctg gac cgc cca acc tgg cag
 gtg agc aat gct gtg cac cag tcc 576 Pro Leu Asp Arg Pro Thr Trp Gln Val Ser
 Asn Ala Val His Gln Ser 180 185 190 tct gcc tac cac gcc cct ctc cag ctg caa
 tcc tcc atc gcc gag aca 624 Ser Ala Tyr His Ala Pro Leu Gln Leu Gln Ser Ser
 Ile Ala Glu Thr 195 200 205 tct ggt tta gaa aat att ttt gta gga ggc tgg ggt
 tta ggg gat acc 672 Ser Gly Leu Glu Asn Ile Phe Val Gly Gly Ser Gly Leu Gly
 Asp Thr 210 215 220 ggc ggc gag aac atc gag ctg acc tac ttc ggc tcc ccc cgc
 acc agc 720 Gly Gly Glu Asn Ile Glu Leu Thr Tyr Phe Gly Ser Pro Arg Thr Ser
 225 230 235 240 acc ccc cgc tcc atc gcc tcc aag tcc cgc ggc atc ctg aac tgg
 ttc 768 Thr Pro Arg Ser Ile Ala Ser Lys Ser Arg Gly Ile Leu Asn Trp Phe 245
 250 255 agc aag cgg tac tac acc cag gtg ccc acc gaa gat ccc gaa gtg ttc 816
 Ser Lys Arg Tyr Tyr Thr Gln Val Pro Thr Glu Asp Pro Glu Val Phe 260 265 270
 tcc tcc cag acc ttc gcc aac ccc ctg tac gag gcc gag ccc gcc gtg 864 Ser Ser
 Gln Thr Phe Ala Asn Pro Leu Tyr Glu Ala Glu Pro Ala Val 275 280 285 ctg aag
 ggc cct agc ggc cgc gtg ggc ctg tcc cag gtg tac aag cct 912 Leu Lys Gly Pro
 Ser Gly Arg Val Gly Leu Ser Gln Val Tyr Lys Pro 290 295 300 gat acc ctg acc
 aca cgt agc ggc aca gag gtg ggc ccc cag ctg cat 960 Asp Thr Leu Thr Thr Arg
 Ser Gly Thr Glu Val Gly Pro Gln Leu His 305 310 315 320 gtg agg tac tcc ctg
 tcc acc atc cat gag gat gtg gag gct atc ccc 1008 Val Arg Tyr Ser Leu Ser Thr
 Ile His Glu Asp Val Glu Ala Ile Pro 325 330 335 tac acc gtg gat gag aac acc
 cag ggc ctg gcc ttc gtg ccc ctg cat 1056 Tyr Thr Val Asp Glu Asn Thr Gln Gly
 Leu Ala Phe Val Pro Leu His 340 345 350 gag gag cag gcc gcc ttc gag gag atc
 gag ctc gac gat ttc agc gag 1104 Glu Glu Gln Ala Gly Phe Glu Glu Ile Glu Leu
 Asp Asp Phe Ser Glu 355 360 365 acc cat cgc ctg ctg ccc cag aac acc tcc tcc
 acc ccc gtg ggc agc 1152 Thr His Arg Leu Leu Pro Gln Asn Thr Ser Ser Thr Pro
 Val Gly Ser 370 375 380 ggc gtg cgc aga agc ctg atc cct acc cga gag ttc agc
 gcc acc cgg 1200 Gly Val Arg Arg Ser Leu Ile Pro Thr Arg Glu Phe Ser Ala Thr
 Arg 385 390 395 400 cct acc ggc gtg gtg acc tac ggc tcc ccc gac acc tac tcc
 gct agc 1248 Pro Thr Gly Val Val Thr Tyr Gly Ser Pro Asp Thr Tyr Ser Ala Ser
 405 410 415 ccc gtg acc gac cct gat tct acc tct cct agc ctg gtg atc gac gac
 1296 Pro Val Thr Asp Pro Asp Ser Thr Ser Pro Ser Leu Val Ile Asp Asp 420 425
 430 acc acc acc acc ccc atc atc atc atc gac ggc cac aca gtg gat ctg 1344 Thr
 Thr Thr Thr Pro Ile Ile Ile Ile Asp Gly His Thr Val Asp Leu 435 440 445 tac
 agc agc aac tac acc ctg cat ccc tcc ctg ctg agg aag cgc aag 1392 Tyr Ser Ser
 Asn Tyr Thr Leu His Pro Ser Leu Leu Arg Lys Arg Lys 450 455 460 aag cgc aag
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 <223> OTHER INFORMATION: Description of Artificial Sequence Bovine
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 Arg Thr Cys Lys Gln Ala Gly Thr Cys Pro Pro Asp Val Ile Arg Lys 20 25 30 Val
 Glu Gly Asp Thr Ile Ala Asp Lys Ile Leu Lys Phe Gly Gly Leu 35 40 45 Ala Ile

Tyr Leu Gly Gly Leu Gly Ile Gly Thr Trp Ser Thr Gly Arg 50 55 60 Val Ala Ala
 Gly Gly Ser Pro Arg Tyr Thr Pro Leu Arg Thr Ala Gly 65 70 75 80 Ser Thr Ser
 Ser Leu Ala Ser Ile Gly Ser Arg Ala Val Thr Ala Gly 85 90 95 Thr Arg Pro Ser
 Ile Gly Ala Gly Ile Pro Leu Asp Thr Leu Glu Thr 100 105 110 Leu Gly Ala Leu
 Arg Pro Gly Val Tyr Glu Asp Thr Val Leu Pro Glu 115 120 125 Ala Pro Ala Ile
 Val Thr Pro Asp Ala Val Pro Ala Asp Ser Gly Leu 130 135 140 Asp Ala Leu Ser
 Ile Gly Thr Asp Ser Ser Thr Glu Thr Leu Ile Thr 145 150 155 160 Leu Leu Glu
 Pro Glu Gly Pro Glu Asp Ile Ala Val Leu Glu Leu Gln 165 170 175 Pro Leu Asp
 Arg Pro Thr Trp Gln Val Ser Asn Ala Val His Gln Ser 180 185 190 Ser Ala Tyr
 His Ala Pro Leu Gln Leu Gln Ser Ser Ile Ala Glu Thr 195 200 205 Ser Gly Leu
 Glu Asn Ile Phe Val Gly Gly Ser Gly Leu Gly Asp Thr 210 215 220 Gly Gly Glu
 Asn Ile Glu Leu Thr Tyr Phe Gly Ser Pro Arg Thr Ser 225 230 235 240 Thr Pro
 Arg Ser Ile Ala Ser Lys Ser Arg Gly Ile Leu Asn Trp Phe 245 250 255 Ser Lys
 Arg Tyr Tyr Thr Gln Val Pro Thr Glu Asp Pro Glu Val Phe 260 265 270 Ser Ser
 Gln Thr Phe Ala Asn Pro Leu Tyr Glu Ala Glu Pro Ala Val 275 280 285 Leu Lys
 Gly Pro Ser Gly Arg Val Gly Leu Ser Gln Val Tyr Lys Pro 290 295 300 Asp Thr
 Leu Thr Thr Arg Ser Gly Thr Glu Val Gly Pro Gln Leu His 305 310 315 320 Val
 Arg Tyr Ser Leu Ser Thr Ile His Glu Asp Val Glu Ala Ile Pro 325 330 335 Tyr
 Thr Val Asp Glu Asn Thr Gln Gly Leu Ala Phe Val Pro Leu His 340 345 350 Glu
 Glu Gln Ala Gly Phe Glu Glu Ile Glu Leu Asp Asp Phe Ser Glu 355 360 365 Thr
 His Arg Leu Leu Pro Gln Asn Thr Ser Ser Thr Pro Val Gly Ser 370 375 380 Gly
 Val Arg Arg Ser Leu Ile Pro Thr Arg Glu Phe Ser Ala Thr Arg 385 390 395 400
 Pro Thr Gly Val Val Thr Tyr Gly Ser Pro Asp Thr Tyr Ser Ala Ser 405 410 415
 Pro Val Thr Asp Pro Asp Ser Thr Ser Pro Ser Leu Val Ile Asp Asp 420 425 430
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 Ile Leu Val 1 5 10 15 gaa ctg gat ggc gat gtg aat ggg cac aaa ttt tct gtc agc
 gga gag 96 Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu 20
 25 30 ggt gaa ggt gat gcc aca tac gga aag ctc acc ctg aaa ttc atc tgc 144 Gly
 Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys 35 40 45 acc act
 gga aag ctc cct gtg cca tgg cca aca ctg gtc act acc ttc 192 Thr Thr Gly Lys
 Leu Pro Val Pro Trp Pro Thr Leu Val Thr Phe 50 55 60 tct tat ggc gtg cag
 tgc ttt tcc aga tac cca gac cat atg aag cag 240 Ser Tyr Gly Val Gln Cys Phe
 Ser Arg Tyr Pro Asp His Met Lys Gln 65 70 75 80 cat gac ttt ttc aag agc gcc
 atg ccc gag ggc tat gtg cag gag aga 288 His Asp Phe Phe Lys Ser Ala Met Pro
 Glu Gly Tyr Val Gln Glu Arg 85 90 95 acc atc ttt ttc aaa gat gac ggg aac tac
 aag acc cgc gct gaa gtc 336 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr
 Arg Ala Glu Val 100 105 110 aag ttc gaa ggt gac acc ctg gtg aat aga atc gag
 ctg aag ggc att 384 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
 Gly Ile 115 120 125 gac ttt aag gag gat gga aac att ctc ggc cac aag ctg gaa
 tac aac 432 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140 tat aac tcc cac aat gtg tac atc atg gcc gac aag caa aag aat ggc
 480 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly 145 150
 155 160 atc aag gtc aac ttc aag atc aga cac aac att gag gat gga tcc gtg 528
 Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val 165 170 175
 cag ctg gcc gac cat tat caa cag aac act cca atc ggc gac ggc cct 576 Gln Leu
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro 180 185 190 gtg ctc

ctc cca gac aac cat tac ctg tcc acc cag tct gcc ctg tct 624 Val Leu Leu Pro
 Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser 195 200 205 aaa gat ccc aac
 gaa aag aga gac cac atg gtc ctg ctg gag ttt gtg 672 Lys Asp Pro Asn Glu Lys
 Arg Asp His Met Val Leu Leu Glu Phe Val 210 215 220 acc gct gct ggg atc aca
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Detailed Description Paragraph Table - DETL:

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 <400> SEQUENCE: 10 Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
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 Gly Glu 20 25 30 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 Cys 35 40 45 Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60 Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln 65
 70 75 80 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85
 90 95 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val 100 105
 110 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 120
 125 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn 130 135
 140 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly 145 150
 155 160 Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val 165
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 185 190 Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser 195
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 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu 20 25 30 ggg gaa ggg gat
 gca aca tat ggg aaa cta aca cta aaa ttt ata tgc 144 Gly Glu Gly Asp Ala Thr
 Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys 35 40 45 aca aca ggg aaa cta cct gtg
 cca tgg cct aca cta gtg aca aca ttt 192 Thr Thr Gly Lys Leu Pro Val Pro Trp
 Pro Thr Leu Val Thr Thr Phe 50 55 60 agt tat ggg gtg caa tgc ttt agt aga tat
 cct gat cat atg aaa caa 240 Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp
 His Met Lys Gln 65 70 75 80 cat gat ttt ttt aaa agt gca atg ccc gag ggg tat
 gtg caa gaa aga 288 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
 Glu Arg 85 90 95 aca ata ttt ttt aaa gat gat ggg aat tat aaa aca aga gca gaa
 gtc 336 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val 100
 105 110 aaa ttt gaa ggg gat aca cta gtg aat aga ata gag ctc aaa ggg ata 384
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 Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn 130 135 140 tat aat
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 Gln Asn Thr Pro Ile Gly Asp Gly Pro 180 185 190 gtg cta cta cct gat aac cat
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 Ser Thr Gln Ser Ala Leu Ser 195 200 205 aaa gat cct aat gaa aaa aga gat cat
 atg gtg cta ctc gag ttt gtg 672 Lys Asp Pro Asn Glu Lys Arg Asp His Met Val
 Leu Leu Glu Phe Val 210 215 220 aca gca gca ggg ata aca cat ggg atg gat gaa
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 Ile Leu Val 1 5 10 15 Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
 Gly Glu 20 25 30 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 Cys 35 40 45 Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60 Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln 65
 70 75 80 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85
 90 95 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val 100 105
 110 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 120
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 185 190 Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser 195
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FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence
Oligonucleotide specific for Leu(CTT)

US-PAT-NO: 6379944

DOCUMENT-IDENTIFIER: US 6379944 B1

TITLE: Mammalian cell lines expressing bovine adenovirus functions

DATE-ISSUED: April 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|------------|-------|----------|---------|
| Mittal; Suresh K. | Saskatoon | N/A | N/A | CA |
| Graham; Frank L. | Hamilton | N/A | N/A | CA |
| Prevec; Ludvik | Burlington | N/A | N/A | CA |
| Babiuk; Lorne A. | Saskatoon | N/A | N/A | CA |
| Tikoo; Suresh Kumar | Saskatoon | N/A | N/A | CA |

APPL-NO: 09/ 435242

DATE FILED: November 5, 1999

PARENT-CASE:

REFERENCE TO RELATED APPLICATIONS This is a continuation of allowed U.S. patent application Ser. No. 08/815,927; filed Mar. 13, 1997; now U.S. Pat. No. 6,086,890; which is a continuation of U.S. patent application Ser. No. 08/164,292; filed Dec. 9, 1993; now U.S. Pat. No. 5,820,868.

US-CL-CURRENT: 435/235.1; 435/325 ; 435/455

ABSTRACT:

The present invention provides novel recombinant mammalian cell lines expressing bovine adenovirus functions. In one embodiment, mammalian cell lines stably transformed with bovine adenovirus E1 sequences express bovine adenovirus E1 gene products and are useful for the replication of bovine adenovirus vectors having deletions and/or insertions in the E1 region. Such vector/cell line combinations are useful for synthesis of recombinant polypeptides, preparation of subunit vaccines, and in gene therapy.

28 Claims, 57 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 50

----- KWIC -----

Detailed Description Text - DETX:

A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, viral DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Detailed Description Text - DETX:

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a viral gene, the gene will usually be flanked by DNA that does not flank the viral gene in the genome of the source virus or virus-infected cells. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

Detailed Description Text - DETX:

Luciferase was expressed as an active enzyme as determined by luciferase assays using extracts from MDBK cells-infected with BAV3-Luc (see FIG. 13). The luciferase gene without any exogenous regulatory sequences was inserted into E3 of the BAV3 genome, therefore, there was a possibility of luciferase expression as a fusion protein with part of an E3 protein if the luciferase gene was in the same frame. Such as, F1 and F3 which represent open reading frames (ORFs) for E3 proteins (FIG. 15) or the fusion protein may arise due to recognition of an upstream initiation codon in the luciferase ORF. To explore this possibility we sequenced the DNA at the junction of the luciferase gene and the BAV3 sequences with the help of a plasmid, pSM51-Luc and a synthetic primer design to bind luciferase coding sequences near the initiation codon (data not shown). The luciferase coding region fell in frame F2. The luciferase initiation codon was the first start codon in this frame, however, the ORF started at 84 nucleotides upstream of the luciferase start codon. To further confirm that luciferase protein is of the same molecular weight as purified firefly luciferase, unlabeled mock-infected, wt BAV3-infected or BAV3-Luc-infected MDBK cell extracts were reacted with an anti-luciferase antibody in a Western blot (FIG. 16). A 62 kDa polypeptide band was visible in the BAV3-Luc (lane 3 and 4)-infected cell extracts which were of the same molecular weight as pure firefly luciferase (lane 5). We are not sure whether a band of approximately 30 kDa which also reacted with the anti-luciferase antibody in lanes 3 and 4 represented a degraded luciferase protein.

US-PAT-NO: 6331527

DOCUMENT-IDENTIFIER: US 6331527 B1

TITLE: Promoter smooth muscle cell expression

DATE-ISSUED: December 18, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|-----------|-------|----------|---------|
| Parmacek; Michael S. | Bryn Mawr | PA | N/A | N/A |
| Solway; Julian | Glencoe | IL | N/A | N/A |

APPL-NO: 09/ 431349

DATE FILED: November 1, 1999

PARENT-CASE:

This is a divisional of co-pending application Ser. No. 08/726,807, filed Oct. 7, 1996, which claims the benefit of priority of U.S. Provisional Application No. 60/004,868, filed Oct. 5, 1995.

US-CL-CURRENT: 514/44; 435/455 ; 435/456 ; 536/24.1 ; 623/1.13 ; 623/1.41

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

75 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the **gene expression is down-regulated to non-detectable levels in "synthetic SMCs"** located within atherosclerotic plaques (Shanahan et al.,

1994), suggests that both positive and negative **regulatory** mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive **regulatory** factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the **luciferase** activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmYY1 expression plasmid that encodes a **mutant** YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a **luciferase** reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been **mutagenized** to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative **regulatory** factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive **regulatory** factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6319716

DOCUMENT-IDENTIFIER: US 6319716 B1

TITLE: Bovine adenovirus type 3 genome and vector systems derived therefrom

DATE-ISSUED: November 20, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------------|--------------|-------|----------|---------|
| Tikoo; Suresh Kumar | Saskatoon | N/A | N/A | CA |
| Babiuk; Lorne A. | Saskatoon | N/A | N/A | CA |
| Reddy; Police Seshidhar | Gaithersburg | MD | N/A | N/A |
| Zakhartchouk; Alexandre | Saskatoon | N/A | N/A | CA |
| Baxi; Mohit | Saskatoon | N/A | N/A | CA |

APPL-NO: 09/ 103330

DATE FILED: June 23, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of U.S. patent application Ser. No. 08/880,234, filed Jun. 23, 1997, now abandoned, the disclosure of which is hereby incorporated herein by reference.

US-CL-CURRENT: 435/471; 424/199.1 ; 424/93.2 ; 435/235.1 ; 435/320.1 ; 435/472 ; 435/475 ; 435/477

ABSTRACT:

The present invention provides the complete nucleotide sequence of a bovine adenovirus. The invention further provides bovine adenovirus vectors and expression systems which can be used, among other things, for insertion of foreign sequences, for provision of DNA control sequences including transcriptional and translational regulatory sequences, for diagnostic purposes to detect the presence of viral nucleic acids or proteins encoded by these regions in a subject or biological sample, for provision of immunogenic polypeptides or fragments thereof, for vaccines and for gene therapy. Cell lines comprising the vectors of the invention, and methods for making bovine adenovirus vectors are also provided.

35 Claims, 103 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 94

----- KWIC -----

Detailed Description Text - DETX:

A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate **regulatory** sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA **sequences from eucaryotic (e.g., mammalian) DNA, viral DNA, and even synthetic DNA sequences.** A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Detailed Description Text - DETX:

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a viral gene, the gene will usually be flanked by DNA that does not flank the viral gene in the genome of the source virus or virus-infected cells. Another example of the heterologous coding **sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences** having codons different from the native gene). Allelic variation or naturally occurring **mutational** events do not give rise to a heterologous region of DNA, as used herein.

Detailed Description Text - DETX:

Luciferase was expressed as an active enzyme as determined by luciferase **assays** using extracts from MDBK cells-infected with BAV3-Luc (see FIG. 13). The luciferase **gene without** any exogenous regulatory **sequences** was inserted into E3 of the BAV3 genome, therefore, there was a possibility of luciferase **expression** as a fusion protein with part of an E3 protein if the luciferase **gene was** in the same frame (such as F1 and F3 which represent open reading frames (ORFs) for E3 proteins, FIG. 15) or the fusion protein may arise due to recognition of an upstream initiation codon in the luciferase **ORF**. To explore this possibility we sequenced the DNA at the junction of the luciferase **gene and the BAV3 sequences with the help of a plasmid, pSM51-Luc and a synthetic primer designed to bind luciferase coding sequences near the** initiation codon (data not shown). The luciferase **coding region** fell in frame F2. The luciferase **initiation** codon was the first start codon in this frame, however, the ORF started 84 nucleotides upstream of the luciferase **start codon**. To further confirm that luciferase **protein** is of the same molecular weight as purified firefly luciferase, **unlabeled** mock-infected, wt BAV3-infected or BAV3-Luc-infected MDBK cell extracts were reacted with an anti-luciferase **antibody** in a Western blot (FIG. 16). A 62 kDa polypeptide band was visible in the BAV3-Luc (lanes 3 and 4)-infected cell extracts which was of the same molecular weight as pure firefly luciferase **(lane 5)**. A band of approximately

30 kDa also reacted with the anti-luciferase antibody (lanes 3 and 4) and may represent a degraded luciferase protein.

US-PAT-NO: 6297221

DOCUMENT-IDENTIFIER: US 6297221 B1

TITLE: Method for promoting angiogenesis with a nucleic acid construct comprising an SM22.alpha.0 promoter

DATE-ISSUED: October 2, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|---------|-------|----------|---------|
| Parmacek; Michael S. | Chicago | IL | N/A | N/A |
| Solway; Julian | Glencoe | IL | N/A | N/A |

APPL-NO: 09/ 225670

DATE FILED: January 5, 1999

PARENT-CASE:

This is a divisional of application Ser. No. 08/726,807 filed Oct. 7, 1996, now U.S. Pat. No. 6,090,618, from U.S. Provisional Application No. 60,004,868, filed Oct. 5, 1995.

US-CL-CURRENT: 514/44

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

15 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the gene expression is down-regulated to non-detectable levels

in "**synthetic** SMCs" located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative **regulatory** mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive **regulatory** factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY 1 in C2C 12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the **luciferase** activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmYY1 expression plasmid that encodes a **mutant** YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a **luciferase** reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been **mutagenized** to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative **regulatory** factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive **regulatory** factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6291211

DOCUMENT-IDENTIFIER: US 6291211 B1

TITLE: Promoter for smooth muscle cell expression

DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|---------|-------|----------|---------|
| Parmacek; Michael S. | Chicago | IL | N/A | N/A |
| Solway; Julian | Glencoe | IL | N/A | N/A |

APPL-NO: 09/ 431414

DATE FILED: November 1, 1999

PARENT-CASE:

This is a divisional of application Ser. No. 08/726,807, filed Oct. 7, 1996, now U.S. Pat. No. 6,090,618, which claims the benefit of priority to U.S. Provisional Application No. 60/004,868 filed Oct. 5, 1995.

US-CL-CURRENT: 435/69.1; 435/455 ; 435/456 ; 435/91.3 ; 514/44

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

32 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the gene expression is down-regulated to non-detectable levels in "synthetic SMCs" located within atherosclerotic plaques (Shanahan et al.,

1994), suggests that both positive and negative **regulatory** mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive **regulatory** factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the **luciferase** activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNA_mYY1 expression plasmid that encodes a **mutant** YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a **luciferase** reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been **mutagenized** to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative **regulatory** factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive **regulatory** factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6287765

DOCUMENT-IDENTIFIER: US 6287765 B1

TITLE: Methods for detecting and identifying single molecules

DATE-ISSUED: September 11, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|-----------|-------|----------|---------|
| Cubicciotti; Roger S. | Montclair | NJ | N/A | N/A |

APPL-NO: 09/ 081930

DATE FILED: May 20, 1998

US-CL-CURRENT: 435/6; 435/91.2 ; 536/22.1 ; 536/23.1 ; 536/24.3 ; 536/24.5

ABSTRACT:

Multimolecular devices and drug delivery systems prepared from synthetic heteropolymers, heteropolymeric discrete structures, multivalent heteropolymeric hybrid structures, aptameric multimolecular devices, multivalent imprints, tethered specific recognition devices, paired specific recognition devices, nonaptameric multimolecular devices and immobilized multimolecular structures are provided, including molecular adsorbents and multimolecular adherents, adhesives, transducers, switches, sensors and delivery systems. Methods for selecting single synthetic nucleotides, shape-specific probes and specifically attractive surfaces for use in these multimolecular devices are also provided. In addition, paired nucleotide-nonnucleotide mapping libraries for transposition of selected populations of selected nonoligonucleotide molecules into selected populations of replicatable nucleotide sequences are described.

27 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

The term "conjugate" means two or more molecules, at least one being a selected molecule, attached to one another in an irreversible or pseudoirreversible manner, typically by covalent and/or specific attachment. A first selected molecule may be conjugated to a second molecule or to a nucleic acid sequence either indirectly, e.g., through an intervening spacer arm, group, molecule, bridge, carrier, or specific recognition partner, or directly, i.e., without an

intervening spacer arm, group, molecule, bridge, carrier or specific recognition partner, advantageously by direct covalent attachment. A selected molecule may be conjugated to a nucleotide via hybridization, provided the selected molecule is tagged with an oligonucleotide complementary to a selected nucleic acid sequence comprising the nucleotide. Other noncovalent means for conjugation of nucleotide and nonnucleotide molecules include, e.g., ionic bonding, hydrophobic interactions, ligand-nucleotide binding, chelating agent/metal ion pairs or specific binding pairs such as avidin/biotin, streptavidin/biotin, anti-fluorescein/fluorescein, anti-2,4-dinitrophenol (DNP)/DNP, anti-peroxidase/oxidase, anti-digoxigenin/digoxigenin or, more generally, receptor/ligand. For example, a reporter molecule such as alkaline phosphatase, horseradish peroxidase, β -galactosidase, urease, **luciferase**, rhodamine, fluorescein, phycoerythrin, luminol, isoluminol, an acridinium ester or a fluorescent microsphere which is attached, e.g., for labeling purposes, to a selected molecule or selected nucleic acid sequence using avidin/biotin, streptavidin/biotin, anti-fluorescein/fluorescein, anti-peroxidase/oxidase, anti-DNP/DNP, anti-digoxigenin/digoxigenin or receptor/ligand (i.e., rather than being directly and covalently attached) is said to be conjugated to the selected molecule or selected nucleic acid sequence by means of a specific binding pair. The term "conjugate" does not include an unmodified sequence of nucleotides, referred to herein as a molecule, nucleic acid, nucleotide, defined sequence segment, nucleotide sequence or oligonucleotide. However, oligonucleotides, aptamers, **synthetic heteropolymers, defined sequence** segments and selected nucleic acid sequences may be referred to as conjugates if a nonnucleotide molecule, group or moiety (e.g., biotin, digoxigenin, fluorescein, rhodamine) is introduced as a nucleotide analog, modified nucleotide or nucleoside triphosphate before, during or after nucleic acid synthesis.

Brief Summary Text - BSTX:

"Selected nucleic acid **sequences**" include, but are not limited to, **defined sequence segments of synthetic** heteropolymers and discrete structures, heteropolymeric, aptameric and nonaptameric nucleotide-based devices, oligonucleotides, and RNA, DNA or denatured RNA or DNA sequences, including wild-type, **mutant** and recombinant biological nucleic acid **sequences; biological, recombinant, engineered and synthetic** nucleic acids comprising specific or catalytic recognition sites or properties, e.g., aptamers, catalytic DNA, ribozymes, nucleic acid ligands, nucleic acid receptors, nucleic acid antibodies and nucleic acid molecules capable of participating in specific recognition, catalytic and enzymatic reactions; genomic, plasmid, cellular and transcribed or complementary nucleic acids, including DNA, cDNA and RNA; natural and **synthetic coding, noncoding, initiation, termination, promoter and regulatory sequences, including natural, synthetic**, native or nonnative biological recognition sites and therapeutic targets; natural and synthetic oligonucleotides with defined topology, secondary or tertiary structure or three-dimensional shape, including rolling and circular nucleic acids, nucleic acid loops, stems, bulges, knots, pseudoknots, polygons, spheres, pyramids, cubes, and higher order three-dimensional shapes; immobilized, conjugated, labeled and insolubilized nucleic acids, including nucleic acids hybridized or specifically bound to other soluble, insoluble, immobilized, conjugated or labeled nucleic acids; nucleic acid probes, targets and templates; sense,

antisense and antigene nucleic acid strands; conjugated defined sequence segments and conjugated oligonucleotides, including oligonucleotides that are internally conjugated to provide closed-loop, single-ended or double-ended loop structures; branched, branched-chain, branched-comb, multi-chain and "Christmas tree" oligonucleotides; nucleic acid dendrons, dendrimers and nucleic acid conjugates formed by coulombic, affinity-based or covalent interactions with dendrons, dendrimers and other branched and hyperbranched structures; nucleotides comprising or capable of forming single-stranded, double-stranded, partially single-stranded, partially double-stranded, heteroduplex, triplex, quadruplex, chimeric and hybrid structures comprising natural or synthetic RNA, DNA or oligonucleotides comprising nucleotide analogs, derivatized nucleotides, nucleosides, nucleoside phosphates or backbone modifications. Selected nucleic acid **sequences hybridized to bifunctional synthetic** heteropolymers do not include unconjugated primers that hybridize to fixed primer-annealing sequences of aptamers selected from mixtures of random-sequence nucleic acids.

Brief Summary Text - BSTX:

The synthetic heteropolymers of the instant invention are not derived, selected or copied from wild-type biological nucleic acid molecules, sequences or groups of contiguous sequences, nor are they derived, isolated, selected or copied from heretofore known **mutants**, genetic variants or nucleic acid molecules or sequences therefrom. At least one defined **sequence segment of each synthetic** heteropolymer or multivalent heteropolymeric hybrid structure of the instant invention is not only capable of specifically binding a nonoligonucleotide molecule, but is also synthetic. When used to describe a defined **sequence segment, synthetic means nonnaturally occurring, i.e., the defined sequence segment is not heretofore known to occur in nature (sans human biotechnologic intervention) and is not heretofore known to be a biological recognition site.** Biological recognition site means a first biological molecule or nucleic acid sequence that is heretofore known to specifically bind or recognize a second biological molecule or nucleic acid sequence. Unless otherwise specified, artificial and synthetic refer to willful products of human technology. Native, in nature, natural, naturally occurring, biological and organism, by contrast, refer to spontaneously occurring substances or beings that are not willful products of human-directed recombinant or transgenic technologies. In the case of hybrid plants and animals that have been identified and/or perpetuated by cross-breeding, selective breeding, cross-pollination, stem or limb grafting and the like, native, in nature, natural, naturally occurring, biological and organism mean only heretofore known strains. Where the distinction between natural and synthetic is ambiguous, a heretofore known substance, being or strain shall be considered natural for purposes of this disclosure, and a heretofore unknown substance, being or strain shall be considered synthetic.

Brief Summary Text - BSTX:

Effector molecules, also referred to as effector species, effectors and molecular effectors, are molecules, groups of molecules, complexes or conjugates capable of transforming energy into work or work into energy and include, but are not limited to, signal-generating species,

stimulus-response-generating or response molecules, enzymes, synthetic enzymes, drugs, catalytic antibodies, catalysts, contractile proteins, transport proteins, **regulatory** proteins, redox proteins, redox enzymes, redox mediators, cytochromes, electroactive compounds, photoactive compounds, supermolecules and shape-memory structures. Selected nucleic acid **sequences include, but are not limited to, defined sequence segments of synthetic** heteropolymers, MOLECULAR MACHINES, oligonucleotides, and RNA, DNA or denatured DNA sequences, including wild-type, **mutant** and recombinant biological nucleic acid **sequences; biological, engineered and synthetic** nucleic acid ligands, nucleic acid receptors, nucleic acid antibodies and nucleic acid sequences capable of participating in specific binding, catalytic and enzymatic reactions, e.g., aptamers, catalytic DNA and ribozymes; genomic, plasmid, cellular and transcribed or complementary nucleic acids, including DNA, cDNA and RNA; natural and **synthetic coding, noncoding, initiation, termination, promoter and regulatory sequences, including natural, synthetic, native or nonnative biological recognition sequences and therapeutic targets; natural and synthetic** oligonucleotides with defined topology, secondary or tertiary structure or three-dimensional shape, including rolling and circular nucleic acids, nucleic acid loops, stems, bulges, knots, pseudoknots, polygons, spheres, pyramids, cubes, and higher order three-dimensional shapes; immobilized, conjugated, labeled and insolubilized nucleic acids, including nucleic acids hybridized or specifically bound to other soluble, insoluble, immobilized, conjugated or labeled nucleic acids; nucleic acid probes, targets and templates; sense, antisense and antigene nucleic acid strands; conjugated defined sequence segments and conjugated oligonucleotides, including oligonucleotides that are internally conjugated to provide closed-loop or single-ended or double-ended loop structures; branched, branched-chain, branched-comb, multi-chain and Christmas tree oligonucleotides; nucleic acid dendrons, dendrimers and nucleic acid conjugates formed by coulombic, affinity-based or covalent interactions with dendrons, dendrimers and other branched and hyperbranched structures; single-stranded, double-stranded, partially single-stranded, partially double-stranded, heteroduplex, triplex, quadruplex, chimeric and hybrid structures comprising natural or synthetic RNA, DNA or oligonucleotides comprising nucleotide analogs, derivatized nucleotides or nucleoside triphosphates or backbone modifications. A defined sequence segment comprising a first MOLECULAR MACHINE may hybridize or specifically bind to a selected nucleic acid sequence or selected molecule comprising a second MOLECULAR MACHINE, thereby attaching the two MOLECULAR MACHINES. The resulting product, which may be referred to as a single MOLECULAR MACHINE or a pair of MOLECULAR MACHINES, may attach to other MOLECULAR MACHINES by methods described herein, including specific binding, hybridization, site-directed covalent attachment, pseudoirreversible attachment and the like.

Brief Summary Text - BSTX:

The proximity of the selected defined **sequence segments to one another within the synthetic** heteropolymer or multivalent heteropolymeric hybrid structure, which is controlled by the length, composition and three-dimensional structure of the spacer nucleotide and linker oligonucleotide sequences, is such that the binding of a molecule at one defined sequence segment can modulate the affinity of another defined sequence segment for a second nonoligonucleotide molecule.

Modulating the affinity refers to any increase or decrease in the association or dissociation rate constants that characterize the binding between a defined sequence segment and its specific binding partner. The binding of a molecule at one defined sequence segment can also modulate the activity of a molecule bound to another defined sequence segment. Modulating the activity refers to restoration, transduction or elimination in part or in full of the biological, chemical, optical, catalytic, mechanical, electrical or electrochemical activity of a selected molecule or nucleic acid sequence. For example, in a diagnostic assay, specific binding of a nonoligonucleotide molecule such as a receptor or ligand to a second defined **sequence segment of a synthetic heteropolymer may decrease the binding affinity of a first defined sequence** segment for a bound, inactive or partially inactive molecular effector. This results in displacement of the molecular effector and restoration of its activity. Thus, the presence of the selected receptor or ligand may be monitored by measuring activity of the molecular effector. In the case of a selected nucleic acid sequence, activity refers either to catalytic properties (e.g., ribozyme or catalytic DNA activity) or to information content (e.g., coding or **regulatory** properties). Modulation includes effects on catalytic activity, replication, transcription, translation and enzyme-dependent processes such as strand extension, ligation, amplification, and the like.

Brief Summary Text - BSTX:

By positioning molecules so that binding or activity at a first defined sequence segment modulates binding or activity at a second defined **sequence segment, synthetic** heteropolymers of the present invention can be used to functionally couple a first selected molecule or nucleic acid sequence to a second selected molecule or nucleic acid sequence. For example, a first signal-generating molecule such as a fluorophore can be functionally coupled to a second signal-generating molecule such as a second fluorophore (e.g., a donor or acceptor), a light-driven or bioluminescent enzyme (e.g., an ATPase or **luciferase**) or an artificial reaction center (i.e., a molecule capable of photoinduced charge separation).

Brief Summary Text - BSTX:

Heteropolymeric functional coupling of the instant invention does not include the interaction between a ribozyme and its biological recognition site, i.e., the catalytic activity resulting from ribozyme-based recognition and cleavage of a biological nucleic acid sequence. Also outside the scope of the instant invention are ribozymes comprising **synthetic defined sequence** segments that bring the ribozyme catalytic element under allosteric control, i.e., by specific recognition of a selected molecule or selected nucleic acid sequence that **regulates** ribozyme catalytic activity.

Detailed Description Text - DETX:

For most applications, preferred methods for producing synthetic heteropolymers include automated synthesis and biological methods, e.g., using recombinant DNA procedures. However, in some cases it is advantageous to simulate the function

or evaluate the potential utility of a **synthetic heteropolymer using two or more defined sequence** segments which are either readily available or can be conveniently modified for a particular molecular assembly task. In such instances, it may be preferable to prepare a synthetic heteropolymer by less than ideal methods, e.g., by conjugating two defined sequence segments using covalent or pseudoirreversible means. Also, **synthetic heteropolymers comprising defined sequence** segments joined by nonnucleotidic linkages and/or linkers (e.g., nonnucleotide spacer groups, molecules, or polymers) have utility in screening and analytical applications, e.g., to identify compounds or fractions having a desired catalytic activity and/or selectivity. For example, a population, generation or library of enzymes created by site-directed **mutagenesis** or directed in vitro evolution (e.g., random **mutagenesis** plus recombination) can be screened for activity in cleaving a bond connecting two defined sequence segments to which functionally coupled effectors are attached.

US-PAT-NO: 6284743

DOCUMENT-IDENTIFIER: US 6284743 B1

TITLE: Method for modulating smooth muscle cell proliferation

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|---------|-------|----------|---------|
| Parmacek; Michael S. | Chicago | IL | N/A | N/A |
| Solway; Julian | Glencoe | IL | N/A | N/A |

APPL-NO: 09/ 546550

DATE FILED: April 10, 2000

PARENT-CASE:

This is a continuation of application Ser. No. 09/258,367, filed Feb. 26, 1999, now U.S. Pat. No. 6,114,311, which is a divisional of application Ser. No. 08/726,807 filed Oct. 7, 1996, now U.S. Pat. No. 6,090,618, which claims the benefit of priority to U.S. Provisional Application No. 60/004,868, filed Oct. 5, 1995.

US-CL-CURRENT: 514/44; 435/375 ; 435/69.1

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

23 Claims, 14 Drawing figures

Exemplary Claim Number: 1,2

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

The demonstration that the SM22.alpha. gene is expressed at high levels in

medial SMCs, but the **gene expression is down-regulated to non-detectable levels in "synthetic SMCs"** located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative **regulatory** mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME4 binds both SRF (a positive **regulatory** factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p441SM22-luc reporter plasmid into primary rat aortic SMCs and the **luciferase** activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmYY1 expression plasmid that encodes a **mutant** YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a **luciferase** reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been **mutagenized** to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a negative **regulatory** factor. Conversely, the demonstration that over-expression of YY1 increases SM22.alpha. promoter activity would suggest (but not prove) that, as with the c-fos promoter, YY1 acts as a positive **regulatory** factor (Natesan and Gilman, 1995b).

US-PAT-NO: 6225291

DOCUMENT-IDENTIFIER: US 6225291 B1

TITLE: Rod opsin mRNA-specific ribozyme compositions and methods for the treatment of retinal diseases

DATE-ISSUED: May 1, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|-------------|-------|----------|---------|
| Lewin; Alfred S. | Gainesville | FL | N/A | N/A |
| Hauswirth; William W. | Gainesville | FL | N/A | N/A |
| Drenser; Kimberly | Gainesville | FL | N/A | N/A |

APPL-NO: 09/ 063667

DATE FILED: April 21, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application claims priority from provisional applications U.S. Ser. No. 60/046,147, filed May 9, 1997; and U.S. Ser. No. 60/044,492, filed Apr. 21, 1997.

US-CL-CURRENT: 514/44; 435/320.1 ; 435/375 ; 435/456 ; 435/91.31 ; 536/23.1 ; 536/24.5

ABSTRACT:

The subject invention provides materials and methods for efficient, specific reduction or elimination of unwanted mRNA. These materials and methods can be used in therapies for retinal diseases. In one embodiment, ribozymes which degrade mutant mRNA are used to treat retinitis pigmentosa.

62 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

----- KWIC -----

Drawing Description Text - DRTX:

FIGS. 2a and 2b show the construction of plasmids used according to the subject invention. These figures show a schematic diagram of the plasmid DNA constructs used to make rAAV viruses mOp-lacZ (a) and mOp-gfp (b). TR, 145 bp

AAV terminal repeat sequence, mOp, 472 bp murine rod opsin regulatory sequence from +86 to -388, SD/SA, 180 bp SV40 late viral protein gene 16S/19S splice donor and acceptor signal, lacZ; coding sequence for the bacterial lacZ gene; gfp, coding sequence for the synthetic green fluorescence gene; pA, pA1 and pA2, polyadenylation signals; Epo, a tandem repeat of the polyoma virus enhancer region (bases 5210-5274); Ptk, thymidine kinase promoter of herpesvirus (bases 92-218); neor, coding sequence of the neomycin resistance gene, Tn5 (bases 1555-2347) (Zolotukhin, S., M. Potter, W. Hauswirth, J. Guy, N. Muzyczka [1996] J. Virol. 70:4646-4654).

Detailed Description Text - DETX:

In one embodiment the subject invention concerns synthetic genes for several ribozymes. These ribozymes recognize the nucleotide change causing the P23H mutation in one form of ADRP and the S334ter mutation in another. Genes have been constructed which encode several ribozymes having the ability to specifically destroy target RNAs for mutant retina proteins. Specifically, FIG. 3 shows a ribozyme for the destruction of RNA having the S334 mutation. FIGS. 4, 5, and 6 show ribozymes which destroy RNA having the P23H mutation. The ribozyme in FIG. 6 is a hammerhead ribozyme (Rz23) which is directed against the human P23H mRNA. With the benefit of the teachings provided herein, the skilled artisan can construct genes encoding ribozymes which destroy mutant RNA molecules associated with human RP or other retinal diseases.

Detailed Description Text - DETX:

A second reporter gene, a synthetic version of the A victoria green fluorescent gene (gfp) (Zolotukhin, S. M. Potter, W. Hauswirth, J. Guy, N. Muzyczka [1996] J. Virol. 70:4646-4654) was used to independently confirm the apparent cell-type specificity of transduction. The same murine rod opsin promoter was used as well as an analogous rAAV vector to construct the mOp-gfp virus (FIG. 2b). Two . μ l of gfp-containing rAAV was injected into the subretinal space of 8 Sprague-Dawley rats. Rats were used in place of mice because the larger eye allowed more reproducible subretinal inoculations. Retinal whole mounts prepared from all eight rat eyes that were injected contained a fluorescent region of superior retina surrounding the site of inoculation. GFP fluorescence typically extended over 10-20% of the retinal area in a radial pattern from the injection site. Immediately surrounding the point of infection, the transduction frequency, as judged by the intensity of GFP fluorescence, was very high, with a continuous positive signal. In transverse sections extending from the central retina to the periphery, beyond a region of apparently saturated GFP fluorescence, the percentage of transduced cells decreased radially with distance from the injection site. GFP-positive cells were easily identifiable as PRs by their specialized shape and location in the retina. Hence, only PR cells appeared to have been transduced, i.e., infected by the rAAV and expressing the gfp passenger gene.

US-PAT-NO: 6114311

DOCUMENT-IDENTIFIER: US 6114311 A

TITLE: Method for modulating smooth muscle cell proliferation

DATE-ISSUED: September 5, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|---------|-------|----------|---------|
| Parmacek; Michael S. | Chicago | IL | N/A | N/A |
| Solway; Julian | Glencoe | IL | N/A | N/A |

APPL-NO: 09/ 258367

DATE FILED: February 26, 1999

PARENT-CASE:

This is a divisional of co-pending application Ser. No. 08/726,807 filed Oct. 7, 1996.

US-CL-CURRENT: 514/44

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

18 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the **gene expression is down-regulated to non-detectable levels in "synthetic SMCs"** located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative **regulatory** mechanisms control

expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive **regulatory** factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al, 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the **luciferase** activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmYY1 expression plasmid that encodes a **mutant** YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a **luciferase** reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been **mutagenized** to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a

US-PAT-NO: 6114148

DOCUMENT-IDENTIFIER: US 6114148 A

TITLE: High level expression of proteins

DATE-ISSUED: September 5, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------|-------------|-------|----------|---------|
| Seed; Brian | Boston | MA | N/A | N/A |
| Haas; Jurgen | Schriesheim | N/A | N/A | DE |

APPL-NO: 08/ 717294

DATE FILED: September 20, 1996

US-CL-CURRENT: 435/91.1; 435/252.3 ; 435/320.1 ; 435/325 ; 435/440 ; 435/69.1 ; 435/69.6 ; 435/91.4 ; 435/91.41

ABSTRACT:

The invention features a synthetic gene encoding a protein normally expressed in a mammalian cell wherein at least one non-preferred or less preferred codon in the natural gene encoding the protein has been replaced by a preferred codon encoding the same amino acid.

18 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

----- KWIC -----

Detailed Description Text - DETX:

To compare the wild-type and **synthetic gp120 coding sequences, the synthetic gp120 coding sequence** was inserted into a mammalian expression vector and tested in transient transfection assays. Several different native gp120 genes were used as controls to exclude variations in expression levels between different virus isolates and artifacts induced by distinct leader sequences. The gp120 HIV IIIb construct used as control was generated by PCR using a Sal1/Xho1 HIV-1 HXB2 envelope fragment as template. To exclude PCR induced **mutations**, a Kpn1/Ear1 fragment containing approximately 1.2 kb of the gene was exchanged with the respective sequence from the proviral clone. The wild-type gp120mn constructs used as controls were cloned by PCR from HIV-1 MN infected C8166 cells (AIDS Repository, Rockville, Md.) and expressed gp120 either with a

native envelope or a CD5 leader sequence. Since proviral clones were not available in this case, two clones of each construct were tested to avoid PCR artifacts. To determine the amount of secreted gp120 semi-quantitatively supernatants of 293T cells transiently transfected by calcium phosphate co-precipitation were immunoprecipitated with soluble CD4:immunoglobulin fusion protein and protein A sepharose.

Detailed Description Text - DETX:

To examine whether regulation by rev is connected to HIV-1 codon usage, the influence of rev on the expression of both native and synthetic gene was investigated. Since regulation by rev requires the rev-binding site RRE in cis, constructs were made in which this binding site was cloned into the 3' untranslated region of both the native and the synthetic gene. These plasmids were co-transfected with rev or a control plasmid in trans into 293T cells, and gp120 expression levels in supernatants were measured semiquantitatively by immunoprecipitation. The procedures used in this experiment are described in greater detail below.

Detailed Description Text - DETX:

As shown in FIG. 5A and FIG. 5B, rev up regulates the native gp120 gene, but has no effect on the expression of the synthetic gp120gene. Thus, the action of rev is not apparent on a substrate which lacks the coding sequence of endogenous viral envelope sequences.

Detailed Description Text - DETX:

The above-described experiment suggest that in fact "envelope sequences" have to be present for rev regulation. In order to test this hypothesis, a synthetic version of the gene encoding the small, typically highly expressed cell surface protein, ratTHY-1 antigen, was prepared. The synthetic version of the ratTHY-1 gene was designed to have a codon usage like that of HIV gp120. In designing this synthetic gene AUUUA sequences, which are associated with mRNA instability, were avoided. In addition, two restriction sites were introduced to simplify manipulation of the resulting gene (FIG. 6). This synthetic gene with the HIV envelope codon usage (rTHY-1env) was generated using three 150 to 170 mer oligonucleotides (FIG. 7). In contrast to the syngp120mn gene, PCR products were directly cloned and assembled in pUC12, and subsequently cloned into pCDM7.

Detailed Description Text - DETX:

Examination of a codon usage table constructed from the native coding sequence of GFP showed that the GFP codons favored either A or U in the third position. The bias in this case favors A less than does the bias of gp120, but is substantial. A synthetic gene was created in which the natural GFP sequence was re-engineered in much the same manner as for gp120 (FIG. 11; SEQ ID NO:40).

In addition, the translation initiation sequence of **GFP** was replaced with sequences corresponding to the translational initiation consensus. The expression of the resulting protein was contrasted with that of the wild type sequence, similarly engineered to bear an optimized translational initiation consensus (FIG. 10B and FIG. 10C). In addition, the effect of inclusion of the **mutation** Ser 65.fwdarw.Thr, reported to improve excitation efficiency of **GFP** at 490 nm and hence preferred for fluorescence microscopy (Heim et al., Nature 373:663, 1995), was examined (FIG. 10D). Codon engineering conferred a significant increase in expression efficiency (an concomitant percentage of cells apparently positive for transfection), and the combination of the Ser 65.fwdarw.Thr **mutation** and codon optimization resulted in a DNA segment encoding a highly visible mammalian marker protein (FIG. 10D).

Detailed Description Text - DETX:

The above-described **synthetic green fluorescent protein coding sequence** was assembled in a similar manner as for gp120 from six fragments of approximately 120 bp each, using a strategy for assembly that relied on the ability of the restriction enzymes BsaI and BbsI to cleave outside of their recognition sequence. Long oligonucleotides were synthesized which contained portions of the coding sequence for **GFP** embedded in flanking sequences encoding EcoRI and BsaI at one end, and BamHI and BbsI at the other end. Thus, each oligonucleotide has the configuration EcoRI/BsaI/GFP fragment/BbsI/BamHI. The restriction site ends generated by the BsaI and BbsI sites were designed to yield compatible ends that could be used to join adjacent **GFP** fragments. Each of the compatible ends were designed to be unique and non-selfcomplementary. The crude synthetic DNA segments were amplified by PCR, inserted between EcoRI and BamHI in pUC9, and sequenced. Subsequently the intact coding sequence was assembled in a six fragment ligation, using insert fragments prepared with BsaI and BbsI. Two of six plasmids resulting from the ligation bore an insert of correct size, and one contained the desired full length sequence. **Mutation** of Ser65 to Thr was accomplished by standard PCR based **mutagenesis**, using a primer that overlapped a unique BssSI site in the synthetic **GFP**.

Detailed Description Text - DETX:

After assembly of the **synthetic gene** it was discovered that there were two undesired residues encoded in the sequence. One was an Arg residue at 749, which is present in the GenBank sequence entry originating from Genentech but is not in the sequence reported by Genentech in the literature. The other was an Ala residue at 146, which should have been Pro. This **mutation** arose at an unidentified step subsequent to the sequencing of the 29 constituent fragments. The Pro749Arg **mutation** was corrected by incorporating the desired change in a PCR primer (ctg ctt ctg acg cgt gct ggg gtg gcg gga gtt; SEQ ID NO:44) that included the MluI site at position 2335 of the sequence below (sequence of HindIII to NotI segment) and amplifying between that primer and a primer (ctg ctg aaa gtc tcc agc tgc; SEQ ID NO:44) 5' to the SgrAI site at 2225. The SgrAI to MluI fragment was then inserted into the expression vector at the cognate sites in the vector, and the resulting correct sequence change verified by sequencing. The Pro146Ala **mutation** was corrected by incorporating the desired

sequence change in an oligonucleotide (ggc agg tgc tta agg aga acg gcc cta tgg cca; SEQ ID NO:46) bearing the AflII site at residue 504, and amplifying the fragment resulting from PCR reaction between that oligo and the primer having sequence cgt tgt tct tca tac gcg tct ggg gct cct cgg ggc (SEQ ID NO:109), cutting the resulting PCR fragment with AflII and AvrII at (residue 989), inserting the corrected fragment into the expression vector and confirming the construction by sequencing.

US-PAT-NO: 6090618

DOCUMENT-IDENTIFIER: US 6090618 A

TITLE: DNA constructs and viral vectors comprising a smooth muscle promoter

DATE-ISSUED: July 18, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|---------|-------|----------|---------|
| Parmacek; Michael S. | Chicago | IL | N/A | N/A |
| Solway; Julian | Glencoe | IL | N/A | N/A |

APPL-NO: 08/ 726807

DATE FILED: October 7, 1996

US-CL-CURRENT: 435/320.1; 536/23.1 ; 536/23.5 ; 536/24.1

ABSTRACT:

Disclosed is a smooth muscle cell specific promoter, the SM22.alpha. gene promoter as well as the murine cDNA and genomic SM22.alpha. nucleic acid sequences. Also disclosed are methods of preventing restenosis following balloon angioplasty and methods of treating asthma based on inhibition of smooth muscle cell proliferation by expressing cell cycle control genes, or contraction inhibiting peptides in smooth muscle cells, under the control of the SM22.alpha. promoter.

62 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

----- KWIC -----

Detailed Description Text - DETX:

The demonstration that the SM22.alpha. gene is expressed at high levels in medial SMCs, but the **gene expression is down-regulated to non-detectable levels in "synthetic SMCs"** located within atherosclerotic plaques (Shanahan et al., 1994), suggests that both positive and negative **regulatory** mechanisms control expression of the SM22.alpha. gene in arterial SMCs. EMSAs (see FIG. 4A and FIG. 4B) revealed that an oligonucleotide probe corresponding to the SME-4 binds both SRF (a positive **regulatory** factor when activated (Johansen and Prywes, 1995)) and YY1 (which can either activate or suppress transcription (Natesan and Gilman, 1995a)). In C2C12 skeletal myoblasts, it has been

demonstrated that YY1 binds CArG box sequences (similar to those present in SME-4) in such a way that it antagonizes SRF action (Gualberto et al., 1992). Moreover, over-expression of YY1 in C2C12 myoblasts has been shown to inhibit differentiation of skeletal myoblasts to terminally differentiated myotubes (Lee et al., 1992). These data are consistent with the hypothesis that protein-protein and protein-DNA interactions that occur at the SM22.alpha. SME-4 nuclear protein binding site serve to activate transcription by binding transcriptional activators such as SRF (and associated proteins), or suppress transcription by binding preferentially to suppressive factors such as YY1. To test this hypothesis, the pcDNAYY1 expression plasmid, which encodes the mouse YY1 protein, is transiently co-transfected with the p-441SM22-luc reporter plasmid into primary rat aortic SMCs and the luciferase activity compared to that of cells transiently co-transfected with the p-441SM22-luc plasmid and the negative control expression plasmid, pcDNA3 (in the same molar ratios). To determine whether the suppressing (or activating) effect of YY1 is dependent upon its DNA-binding activity, the p-441SM22-luc plasmid is transiently co-transfected into primary rat aortic SMCs with the pcDNAmYY1 expression plasmid that encodes a mutant YY1 protein that cannot bind DNA. To determine whether the effect of YY1 on SM22.alpha. promoter activity is dependent on binding directly to the SM22.alpha. promoter (a direct effect versus an indirect effect), the YY1 expression plasmid is co-transfected with a luciferase reporter plasmid under the transcriptional control of the SM22.alpha. promoter which has been mutagenized to abolish YY1 binding activity. Finally, to determine whether YY1-induced suppression of SM22.alpha. promoter activity (if it exists) can be overcome by over-expression of SRF (suggesting a direct antagonism between YY1 and SRF) transient co-transfection studies is performed as described above except that expression plasmids encoding both YY1 and SRF are included and their ratios varied over a range of concentrations. The demonstration that over-expression of YY1 suppresses transcription from the SM22.alpha. promoter would suggest that, as in skeletal muscle cells, YY1 acts as a

US-PAT-NO: 6086890

DOCUMENT-IDENTIFIER: US 6086890 A

TITLE: Bovine adenovirus expression vector system

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|------------|-------|----------|---------|
| Mittal; Suresh K. | Saskatoon | N/A | N/A | CA |
| Graham; Frank L. | Hamilton | N/A | N/A | CA |
| Prevec; Ludvik | Burlington | N/A | N/A | CA |
| Babiuk; Lorne A. | Saskatoon | N/A | N/A | CA |

APPL-NO: 08/ 815927

DATE FILED: March 13, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation of U.S. patent application Ser. No. 08/164,292, filed Dec. 9, 1993, now U.S. Pat. No. 5,820,868.

US-CL-CURRENT: 424/199.1; 424/205.1 ; 424/233.1 ; 424/93.2 ; 435/235.1 ; 435/320.1

ABSTRACT:

The present invention relates novel live bovine adenovirus (BAV) expression vector systems in which part or all of one or both of the early region 1 (E1) and early region 3 (E3) genes are deleted and replaced by a foreign gene or fragment thereof and novel recombinant mammalian cell lines stably transformed with BAV E1 sequences, and therefore, express E1 gene products capable of allowing replication therein of a bovine adenovirus having an E1 deletion replaced by a heterologous nucleotide sequence encoding a foreign gene or fragment thereof and their use in production of (antigenic) polypeptides or fragments thereof for the purpose of live recombinant virus or subunit vaccine or for other therapies.

26 Claims, 56 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 51

----- KWIC -----

Detailed Description Text - DETX:

A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, viral DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Detailed Description Text - DETX:

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a viral gene, the gene will usually be flanked by DNA that does not flank the viral gene in the genome of the source virus or virus-infected cells. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

Detailed Description Text - DETX:

Luciferase was expressed as an active enzyme as determined by luciferase assays using extracts from MDBK cells-infected with BAV3-Luc (see FIG. 13). The luciferase gene without any exogenous regulatory sequences was inserted into E3 of the BAV3 genome, therefore, there was a possibility of luciferase expression as a fusion protein with part of an E3 protein if the luciferase gene was in the same frame. Such as, F1 and F3 which represent open reading frames (ORFs) for E3 proteins (FIG. 15) or the fusion protein may arise due to recognition of an upstream initiation codon in the luciferase ORF. To explore this possibility we sequenced the DNA at the junction of the luciferase gene and the BAV3 sequences with the help of a plasmid, pSM51-Luc and a synthetic primer design to bind luciferase coding sequences near the initiation codon (data not shown). The luciferase coding region fell in frame F2. The luciferase initiation codon was the first start codon in this frame, however, the ORF started at 84 nucleotides upstream of the luciferase start codon. To further confirm that luciferase protein is of the same molecular weight as purified firefly luciferase, unlabeled mock-infected, wt BAV3-infected or BAV3-Luc-infected MDBK cell extracts were reacted with an anti-luciferase antibody in a Western blot (FIG. 16). A 62 kDa polypeptide band was visible in the BAV3-Luc (lane 3 and 4)-infected cell extracts which were of the same molecular weight as pure firefly luciferase (lane 5). We are not sure whether a band of approximately 30 kDa which also reacted with the anti-luciferase antibody in lanes 3 and 4 represented a degraded luciferase protein.

US-PAT-NO: 6020192

DOCUMENT-IDENTIFIER: US 6020192 A

TITLE: Humanized green fluorescent protein genes and methods

DATE-ISSUED: February 1, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|-------------|-------|----------|---------|
| Muzyczka; Nicholas | Gainesville | FL | N/A | N/A |
| Zolotukhin; Sergei | Gainesville | FL | N/A | N/A |
| Hauswirth; William | Gainesville | FL | N/A | N/A |

APPL-NO: 08/ 893327

DATE FILED: July 16, 1997

PARENT-CASE:

This application is a continuation-in-part of United States patent application number 08/588,201 filed Jan. 18, 1997.

US-CL-CURRENT: 435/320.1; 435/235.1 ; 536/23.1 ; 536/23.5

ABSTRACT:

Disclosed are **synthetic and "humanized" versions of green fluorescent protein (GFP) genes** adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such **humanized genes** are also disclosed. In addition, various methods for using the efficient expression of humanized **GFP** in mammalian cells and in animals are described.

11 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

----- KWIC -----

Abstract Text - ABTX:

Disclosed are **synthetic and "humanized" versions of green fluorescent protein (GFP) genes** adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order

to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such humanized genes are also disclosed. In addition, various methods for using the efficient expression of humanized GFP in mammalian cells and in animals are described.

Brief Summary Text - BSTX:

The present invention relates generally to the field of reporter genes and particularly provides improved green fluorescent protein (GFP) genes, constructs and methods of use. The gfp genes disclosed herein are humanized gfp genes adapted for expression in mammalian and human cells by using preferred DNA codons.

Brief Summary Text - BSTX:

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing humanized green fluorescent protein (GFP) genes adapted for expression in mammalian and human cells. The humanized gfp genes of the invention are prepared by incorporating codons preferred for use in human genes into the DNA sequence. Also provided are humanized gfp expression constructs and various methods of using the humanized genes and vectors.

Brief Summary Text - BSTX:

Accordingly, the present invention provides humanized green fluorescent protein (GFP) genes and methods of making and using such genes. As used herein the term a "humanized green fluorescent protein (GFP) gene" means a gene that has been adapted for expression in mammalian and human cells by replacing at least one, and preferably, more than one, and most preferably, a significant number, of jellyfish gfp codons with one or more codons that are more frequently used in human genes.

Brief Summary Text - BSTX:

The humanized genes of the invention are preferably cDNAs, although genomic copies are by no means excluded. The humanized genes are also preferably humanized versions adapted from the A. victoria gfp gene, although other gfp gene sources are, again, not excluded.

Brief Summary Text - BSTX:

In certain embodiments, the present invention provides humanized gfp genes that encode a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2.

Brief Summary Text - BSTX:

In other embodiments, humanized gfp genes will encode GFP variants that are generally based upon the foregoing sequence, but that have certain changes. A particular example is a humanized gene that encodes a GFP with an amino acid sequence of SEQ ID NO:2 in which Serine at position 65 has been replaced by Threonine.

Brief Summary Text - BSTX:

A further example is a humanized gfp gene that encodes a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2 in which Tyrosine at position 66 has been replaced by Histidine.

Brief Summary Text - BSTX:

Another example is a humanized gfp gene that encodes a GFP that has the amino acid sequence of SEQ ID NO:2 in which the chromophore sequence Phe Ser Tyr Gly Val Gln (SEQ ID NO:4) between positions 64 and 69 has been replaced by the sequence Met Gly Tyr Gly Val Leu (SEQ ID NO:5).

Brief Summary Text - BSTX:

Structural equivalents of the humanized gfp genes are also included within the present invention. However, mutants that are truncated by more than one amino acid residue at the amino terminus or more than about 10 or 15 amino acid residues from the carboxyl terminus are not generally considered to be useful in the context of producing a fluorescent protein. The encoded GFP should therefore be a minimum of about 222 amino acids in length, with proteins of about 238 amino acids in length generally being preferred.

Brief Summary Text - BSTX:

Humanized gfp genes wherein at least about 50% or above of the codon positions contain a humanized codon are also contemplated.

Brief Summary Text - BSTX:

Preferred humanized gfp genes of the invention are those genes that contain certain key changes. Examples are genes that comprises at least seven humanized codons from the 10 codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the jellyfish gfp sequence.

Brief Summary Text - BSTX:

Preferably, humanized gfp genes will comprise at least eight, at least nine, or ten, humanized codons from the 10 codons located at codon positions 18, 53, 93,

125, 150, 178, 195, 208, 236 and 224 of the jellyfish **gfp** gene sequence.

Brief Summary Text - BSTX:

Such constructs are exemplified by **humanized genes that comprise any one of the humanized** Leucine codons CTG, CTC or TTG at codon positions 18, 53, 125, 178, 195 and 236 of the **GFP** gene sequence. A further example is a **humanized gfp gene that comprises the humanized** Valine codon GTG at codon positions 93, 150 and 224 of the **GFP** gene sequence. Other examples are **humanized genes that comprise the humanized Serine codon TCT at codon position 208 of the GFP gene** sequence.

Brief Summary Text - BSTX:

The **humanized gfp genes** encompassed by this invention also include those genes that comprises an increased number of GCC or GCT Alanine-encoding codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO: 1.

Brief Summary Text - BSTX:

By the phrase "increased number of codons in comparison to the wild type jellyfish **gene sequence of SEQ ID NO: 1**" is meant that the **humanized sequence** contain an increased number of codons encoding a particular amino acid within the **GFP** coding region that encodes the amino acid sequence of SEQ ID NO:2, or one of the **mutants** or other equivalents described herein, in comparison to those codons encoding the same amino acid that are present within the coding region of the wild type jellyfish gene sequence of SEQ ID NO:1. Thus it will be understood that the term "increased", when used in this context, does not mean the addition of one or more codons to a terminal portion of the coding region, but rather means replacement of an unfavorable codon within the coding region with a codon that is more favorable for translation in a human or mammalian cell.

Brief Summary Text - BSTX:

In light of the definition set forth above, the **humanized gfp genes** of the invention may also be defined as those genes that comprise an increased number of TGC Cysteine-encoding codons; an increased number of GAC Aspartate-encoding codons; an increased number of GAG Glutamate-encoding codons; an increased number of TTC Phenylalanine-encoding codons; an increased number of GGC Glycine-encoding codons; an increased number of CAC Histidine-encoding codons; an increased number of ATC Isoleucine-encoding codons; an increased number of AAG Lysine-encoding codons; an increased number of CTG or CTC Leucine-encoding codons; an increased number of AAC Asparagine-encoding codons; an increased number of CCC or CCT Proline-encoding codons; an increased number of CAG Glutamine-encoding codons; an increased number of CGC, AGG or CGG Arginine-encoding codons; an increased number of AGC or TCC Serine-encoding codons; an increased number of ACC Threonine-encoding codons; an increased

number of GTG or GTC Valine-encoding codons; and/or an increased number of TAC Tyrosine-encoding codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Brief Summary Text - BSTX:

In certain embodiments, the humanized gfp genes may also comprise a TGA termination codon.

Brief Summary Text - BSTX:

Humanized gfp genes may also be defined by comprising a decreased number of certain codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1. "Decreased" in this context also means that the humanized sequence contain a decreased number of codons encoding a particular amino acid within the GFP coding region that encodes the amino acid sequence of SEQ ID NO:2, or a mutant or equivalent thereof, in comparison to those codons encoding the same amino acid that are present within the coding region of the wild type jellyfish gene sequence of SEQ ID NO:1. Thus it will be understood that "decreased" does not in any way reflect the simple deletion of codons from any portion of the coding region, but again refers to replacement of a jellyfish codon with a codon that occurs more frequently in human genes.

Brief Summary Text - BSTX:

Accordingly, humanized gfp genes of the present invention are also be defined as those genes that comprise a decreased number of GCA Alanine-encoding codons; a decreased number of GGU Glycine-encoding codons; a decreased number of CTT, CTA or TTA Leucine-encoding codons; a decreased number of AGA Arginine-encoding codons; a decreased number of AGT, TCA or TCG Serine-encoding codons; or a decreased number of GTT or GTA Valine-encoding codons.

Brief Summary Text - BSTX:

Although not believed to be required, it is currently preferred that the humanized gfp genes should include a Kozak consensus sequence operatively positioned upstream from the humanized gene sequence (i.e., the gene is positioned downstream from the Kozak consensus sequence).

Brief Summary Text - BSTX:

Certain preferred humanized gfp genes will comprise the nucleic acid sequence of SEQ ID NO:3. However, this is by no means limiting and is just one exemplary embodiment of the present invention. Detailed directions as how to make and use many other such humanized gfp genes are included herein. For example, one may refer to the information in Table 2, Table 3 and Table 4 in creating any one of a number of suitable humanized gfp genes.

Brief Summary Text - BSTX:

Virtually any protein- or peptide-encoding DNA sequence, or combinations thereof, may be fused to a humanized gfp sequence in order to encode a fusion protein. This includes DNA sequences that encode targeting peptides, therapeutic proteins, proteins for recombinant expression, proteins to which one or more targeting peptides is attached, protein subunits and the like.

Brief Summary Text - BSTX:

Recombinant vectors and plasmids form another important aspect of the present invention. In such vectors, the humanized gfp gene is positioned under the transcriptional control of a promoter, generally a promoter operative in a mammalian or human cell. "Positioned under the transcriptional control of" means that the humanized gfp sequence is positioned downstream from and under the transcriptional control of the promoter such the promoter is capable of directing expression of the encoded GFP protein in a mammalian or human host cell upon introduction of the vector into such a cell.

Brief Summary Text - BSTX:

The recombinant vectors of the invention will thus generally comprise a humanized gfp reporter gene operatively positioned downstream from a promoter, wherein the promoter is capable of directing expression of the humanized GFP gene in a mammalian or human cell. Preferably the promoter will direct expression of GFP in an amount sufficient to allow GFP detection by detecting the green fluorescence following expression of GFP in the cell. Such promoters are thus "operative" in mammalian and human cells.

Brief Summary Text - BSTX:

Preferred vectors and plasmids will be constructed with at least one multiple cloning site. In certain embodiments, the expression vector will comprise a multiple cloning site that is operatively positioned between a promoter and a humanized gfp gene sequence. Such vectors may be used, in addition to their uses in other embodiments, to create N-terminal fusion proteins by cloning a second protein-encoding DNA segment into the multiple cloning site so that it is contiguous and in-frame with the humanized gfp sequence.

Brief Summary Text - BSTX:

In other embodiments, expression vectors may comprise a multiple cloning site that is operatively positioned downstream from the expressible humanized gfp gene sequence. These vectors are useful, in addition to their uses, in creating C-terminal fusion proteins by cloning a second protein-encoding DNA segment into the multiple cloning site so that it is contiguous and in-frame

with the humanized gfp sequence.

Brief Summary Text - BSTX:

In certain embodiments, the expression vector or plasmid may comprise a humanized GFP reporter gene that has the nucleic acid sequence of SEQ ID NO:3.

Brief Summary Text - BSTX:

Reporter gene expression kits are also provided, which kits generally comprise, in suitable container means, at least one expression vector or plasmid that comprises a humanized GFP gene. The vector or plasmid will generally be one that is capable of expressing GFP in an amount sufficient to allow GFP detection by green fluorescence following expression in a mammalian or human cell.

Brief Summary Text - BSTX:

Recombinant host cells form another aspect of the present invention. Such host cells will generally comprise at least one copy of a humanized GFP gene. Preferred cells for expression purposes will be mammalian and human cells. However, it will be understood that other cell types are not excluded from those of the invention. Accordingly, cells such as bacterial, yeast, fungal, insect, nematode and plant cells are also possible, although such cells are not preferred for expression purposes.

Brief Summary Text - BSTX:

In certain embodiments, the recombinant host cells will preferably incorporate a humanized GFP gene in a manner effective to allow the cell to express, or to be stimulated to express, GFP, most preferably, in an amount sufficient to allow GFP detection by its fluorescence. The recombinant host cell will thus preferably include a humanized GFP gene that was introduced into the cell by means of a recombinant vector.

Brief Summary Text - BSTX:

In certain embodiments, the recombinant host cell will express the humanized GFP gene to produce the encoded GFP protein, preferably, in an amount sufficient to allow GFP detection by its fluorescence. It is contemplated that cells containing as few as about 20 copies of a humanized gfp gene will often express the GFP protein in an amount sufficient to allow GFP detection by green fluorescence. In certain embodiments, cells containing as few as about 10 copies, about 5 copies or even about 1 or 2 copies of a humanized gfp gene will also likely satisfy the desired expression criteria, especially where the humanized gfp gene is a mutant gene. In other embodiments, the recombinant host cells may be capable of expressing a humanized gene in order to produce

detectable GFP protein within a time frame of about 10 hours, and preferably within about 8 hours, and most preferably within about 6 hours or even less.

Brief Summary Text - BSTX:

Cells of primary cell lines that have been established after removing cells from a mammal and culturing the cells for a limited period of time are also included within the cells of the present invention. These cells may be engineered by the hand of man and returned to the same host animal from which they were originally recovered. Such cells that contain a humanized gfp gene fall within the scope of the invention, irrespective of their location.

Brief Summary Text - BSTX:

Naturally, recombinant cells also include those cells that are located within the body of an animal or human subject, as may have been targeted by gene therapy. These cells include all those that comprise at least one copy of a humanized gfp gene or vector, irrespective of the manner in which gene was acquired, e.g., by transfection, infection and the like.

Brief Summary Text - BSTX:

In certain particular embodiments, recombinant host cells that comprise a humanized GFP gene that comprises the nucleic acid sequence of SEQ ID NO:3 are contemplated.

Brief Summary Text - BSTX:

Many methods of using humanized gfp genes are provided by the present invention. The method of labeling or tagging a mammalian or human cell by expressing at least one humanized GFP gene in the cell is central to each of the methods. The humanized gfp gene should preferably produce GFP in an amount sufficient to allow ready detection of GFP in the cell by detecting GFP fluorescence.

Brief Summary Text - BSTX:

Methods of identifying a mammalian or human cell within a population of cells are also provided. Such methods generally first comprise expressing at least one humanized GFP gene in the cell in a manner effective to produce an amount of GFP sufficient to allow GFP detection by fluorescence. The cell is then admixed, or allowed to become naturally admixed, with a population of cells that do not express GFP, following which the cell is identified by means of identifying a GFP-fluorescent cell.

Brief Summary Text - BSTX:

The term "a GFP-fluorescent cell", as used herein, means that a cell expresses a humanized GFP gene in a manner effective to result in the production of the GFP product in an amount sufficient to allow subsequent detection of the cell by detecting green fluorescence from GFP in the cell.

Brief Summary Text - BSTX:

The invention further provides methods for identifying a mammalian or human cell that contains an exogenous DNA segment, which methods generally first comprise introducing into a mammalian or human cell an expression vector comprising a humanized GFP gene operatively linked to an exogenous DNA segment. The cell is then preferably cultured under conditions and for a period of time effective to allow expression of the humanized gfp gene in order to produce an amount of GFP sufficient to allow GFP detection by green fluorescence. Subsequently identifying a cell that contains the exogenous DNA segment is then achieved by identifying a GFP-fluorescent cell.

Brief Summary Text - BSTX:

In certain such embodiments, the expression vector for use in such methods will comprise a first coding region defined as the humanized gfp gene that encodes GFP and will also comprise a second coding region that comprises the exogenous DNA segment. These vectors are generally known as vectors that comprises at least two transcriptional or translational units. Two transcriptional units will naturally include two promoters that direct expression of their respective downstream genes.

Brief Summary Text - BSTX:

Two or more humanized gfp genes, each expressing a GFP protein with different spectral properties, may be detected in a cell in the manner described above. GFP-fluorescent cells, whether expressing one, two or more humanized gfp genes, may be identified by a variety of methods, including microscopy and fluorescence activated cell sorting (FACS).

Brief Summary Text - BSTX:

Further examples of methods of the invention are methods for determining the location of a selected protein within a mammalian or human cell. These methods generally comprise first introducing into a cell an expression vector comprising a contiguous DNA sequence comprising a humanized GFP gene operatively linked to a gene encoding said selected protein. The vector will generally express a fusion protein comprising GFP operatively linked to the selected protein, wherein the fusion protein is produced in amounts sufficient to allow cell detection by detecting the green fluorescence of GFP. One can then identify the location of the selected protein within the cell by identifying the location of the green fluorescence from GFP.

Brief Summary Text - BSTX:

Still further examples of methods of the invention are methods for targeting a protein to a selected location within a mammalian or human cell. These methods generally comprise first introducing into the cell an expression vector comprising a DNA sequence comprising a DNA sequence element that encodes a targeting peptide operatively linked and contiguous with a DNA sequence element of a humanized GFP gene, which is also operatively linked and contiguous with a DNA sequence element that encodes a protein. Such vectors express a fusion protein comprising a targeting peptide operatively linked to GFP and to a protein, wherein the fusion protein is produced in the cell in an amount sufficient to allow cell detection by detecting the GFP fluorescence. The protein is then targeted to a selected location within the cell and the location is confirmed by identifying the location of the green fluorescence.

Brief Summary Text - BSTX:

These methods generally comprises introducing into a cell an expression vector comprising a humanized GFP gene under the control of the candidate promoter and maintaining the cell under conditions effective and for a period of time sufficient to allow expression of the humanized GFP gene by the candidate promoter. "Conditions effective" and "periods of time sufficient" are defined as those conditions and times that would ordinarily result in GFP being produced in an amount sufficient to allow GFP detection by green fluorescence when using a known operative promoter.

Brief Summary Text - BSTX:

A further example of methods for using humanized gfp in the context of promoters are those methods for detecting substances that stimulate transcription from a selected promoter in a mammalian or human cell. Again, one generally introduces into a mammalian or human cell an expression vector comprising a humanized GFP gene under the control of a given promoter. One then exposes the cell to a composition suspected of containing a substance known or suspected to be capable of stimulating transcription from the given promoter. The cell is then cultured or maintained for a period of time that would ordinarily allow an active promoter to stimulate GFP-fusion protein production in an amount sufficient to allow cell detection by detecting the GFP-derived green fluorescence. The subsequent identification of a GFP-fluorescent cell is then indicative of the original presence of a substance that stimulates transcription from the given promoter.

Brief Summary Text - BSTX:

These methods are also suitable for use in vitro and in vivo. In vitro uses allow substances such as toxins and pollutants to be detected by using appropriate promoters within the humanized gfp gene constructs.

Brief Summary Text - BSTX:

As part of gene therapy, it is often necessary to determine gene expression levels in the treated mammalian animal or human subject. The present invention also provides methods for determining such the expression levels. These methods generally comprise expressing in cells of the animal an expression vector comprising a **humanized GFP gene** operatively linked to a selected gene. The expression vector will preferably be either a vector that expresses a **GFP**-fusion protein or a vector in which the **humanized gfp gene** and the selected protein gene each use the same or an equivalent promoter. The promoter will have preferably been shown to result in sufficient **GFP** expression to allow detection in vitro. One then determines the **GFP**-fluorescence in the cells of the animal, wherein the level of **GFP**-fluorescence is indicative of the expression level of the selected gene in the animal.

Brief Summary Text - BSTX:

These methods can be adapted to provide methods for analyzing the expression of a selected gene in different tissues of a mammal or human subject. Such methods generally comprise introducing into the cells of the mammal an expression vector comprising the selected gene under the control of the natural **gene promoter, wherein the gene is operatively linked to a humanized GFP gene**. The vector will preferably express a fusion protein that comprises the encoded gene product operatively linked to **GFP**, the fusion protein being produced in an amount sufficient to allow cell detection by detecting the green fluorescence of **GFP**. After maintaining the mammal under conditions effective and for a period of time sufficient to allow expression of the gene one then analyzes the cells of the tissues of the mammal to detect **GFP**-fluorescent cells, wherein the presence of **GFP**-fluorescent cells in a given tissue is indicative of gene expression in the tissue.

Brief Summary Text - BSTX:

A further example in which the **humanized gfp genes** may be employed is in the recombinant production of **GFP** itself. Such methods of using a **humanized GFP gene simply comprise expressing the humanized gene** in a mammalian or human host cell and collecting the **GFP** expressed by said cell.

Brief Summary Text - BSTX:

(a) preparing a recombinant vector in which a **humanized GFP gene** is positioned under the control of a promoter operative in a mammalian or human cell;

Brief Summary Text - BSTX:

Adaptations of such methods include those wherein the **humanized GFP gene** is

fused to a DNA sequence encoding a protein or peptide of known molecular weight. Expression by the host cell thus results in a **GFP** fusion protein that may be used as a fluorescent molecular weight marker. A range of such fluorescent molecular weight markers could be so-produced to produce a molecular weight determining kit.

Drawing Description Text - DRTX:

FIG. 1A, FIG. 1B and FIG. 1C. Nucleotide sequence of the gfp10 cDNA and the deduced amino acid sequence. Above each codon is the single letter designation for the amino acid. The **mutations** introduced in the gfp.sub.h sequence are shown below the substituted nucleotide of gfp10. The horizontal lines underline overlap regions of mutually priming oligonucleotides used to synthesize the gfp.sub.h cDNA. The sites of the restriction enzymes used to assemble extended pairs of oligonucleotides are shown in bold letters. The codons **mutated** to produce the Ser.sub.65 Thr **mutation**, which produces higher fluorescence yield, and the Tyr.sub.66 His **mutation**, which produces blue fluorescence, are shown in bold. In FIG. 1A, FIG. 1B and FIG. 1C the jellyfish gfp10 nucleotide sequence is SEQ ID NO:1. The deduced amino acid sequence is SEQ ID NO:2. In SEQ ID NO:2, Xaa at position 65 may be Ser or Thr; and Xaa at position 66 may be Tyr or His. The exemplary **humanized gfp sequence** shown below the substituted nucleotide of gfp10 in FIG. 1A, FIG. 1B and FIG. 1C is SEQ ID NO:3. In SEQ ID NO:3, the nucleotides at positions 193, 195 and 196 may be changed in order to encode either Ser or Thr; and either Tyr or His, as above.

Drawing Description Text - DRTX:

For fluorescence microscopy, the inventors increased the sensitivity of the **GFP** reporter **gene system approximately 22 fold for one humanized** construct and at least 45 fold for a second humanized construct. In FACS analyses with **humanized gene** constructs, one construct was at least 32-fold more detectable than the original jellyfish gene, and the other construct was 190-fold more detectable than the original jellyfish gene. When humanized **GFP** is stably integrated as part of the **gfp**-neo cassette of the rAAV provirus in G418-resistant cell lines, a considerable portion of the cells express a visually detectable **GFP**.

Drawing Description Text - DRTX:

In addition, the inventors describe the construction of an Ad shuttle vector, carrying the **humanized GFP reporter gene** under the control of the IRES element. 293 cells infected with recombinant Ad displayed typical CPE and bright green fluorescence. Expression of the **GFP** allowed for quick and easy selection of true recombinant Ad clones, discriminating them from false plaques.

Drawing Description Text - DRTX:

The humanized **GFP** can also be incorporated into other viral and non-viral vector and expression systems. Using the **humanized genes** and vectors of the present invention, efficient transduction and expression of **gfp** gene sequences in mammalian and human cell lines is possible. This is exemplified by gene expression in vivo within neurosensory cells of guinea pig eye, shown herein. The **humanized gfp genes** have many uses, such as in cell sorting by fluorescence-activated cell sorting (FACS), and in human gene therapy.

Drawing Description Text - DRTX:

Green fluorescent protein genes and functional proteins are believed to be present in a variety of organisms, as shown in Table 1. A **gfp** gene from any of the bioluminescent cnidaria and ctenophora that express such **genes can be used as the starting point for preparing a humanized gfp gene** in accordance with the present invention.

Drawing Description Text - DRTX:

It is currently preferred that the **gfp gene sequence from A. victoria be used as the template for creating a humanized gfp gene**, as this is readily available.

Drawing Description Text - DRTX:

Humanized gfp Genes

Drawing Description Text - DRTX:

The foregoing emphasizes the importance of the present invention, the focus of which is to provide for increased **GFP** expression in mammalian and human cells. Each of the **mutants** described above, or indeed any desired **mutant** or a panel of **mutants**, can also be prepared in a humanized background as provided by the present invention. This is because the **humanizing aspects of the invention change the DNA sequence** independently of the protein sequence.

Drawing Description Text - DRTX:

The approach taken by the present inventors is in contrast to the Adams et. al. (1995) method, and addresses the poor translation efficiency of **GFP** mRNA in the human cell environment by using cDNAs that contain base substitutions in order to change the codon usage so that it is more appropriate for expression in mammalian cells. Using such humanized constructs results in green fluorescence in cells that have a low copy number of **humanized gfp genes**, e.g., in the range of less than 10, and even about 1 or 2 when using certain **humanized gfp mutant genes**.

Drawing Description Text - DRTX:

The correlation between the abundance of tRNAs and the occurrence of the respective codons in protein-expressing genes has been described for *E. coli*, yeast and other organisms (Bennetzen and Hall (1982); Grantham et al (1980); Grantham et al. (1981); Ikemura (1981a; 1981b; 1982); Wada et al. (1990)). However, until codon changes are actually made in any given gene, their effects on translation efficiency and overall expression levels cannot be established. This is similar to the situation involving the Kozak sequence, which is not believed to have been particularly helpful in increasing expression of **gfp** in mammalian cells despite expectations. Now that the present inventors have shown that **humanization is effective for gfp gene** expression, the usefulness of the **GFP** technology has been significantly enhanced.

Drawing Description Text - DRTX:

An exemplary **humanized sequence** in accordance with the present invention is represented by SEQ ID NO:3. However, it will be understood that the **humanized sequences** of the present invention are by no means limited to the representative sequence of SEQ ID NO:3. Rather, in light of the following instructions, one of skill in the art will readily be able to prepare a number of different **humanized gfp sequences**.

Drawing Description Text - DRTX:

Although any changes that replace a rarely used jellyfish codon with a codon that is more frequently used in human genes are considered to be useful changes, certain codon changes will naturally be preferred over others. In this regard, the inventors have identified a number of **gfp** codons that are rarely or almost never used in human genes. As discussed below, such codons are the first candidates that should be changed in producing a **humanized gene** in accordance with the present invention.

Drawing Description Text - DRTX:

In making general humanizing changes, codons to be humanized can be identified by those of skill in the art from studying the information presented herein in Tables 2 and in Table 3 and 4. For example, in utilizing the information in Table 2, one would compare the frequency of the jellyfish codon against the frequency of those codons commonly used in human genes, and make any appropriate changes. By way of an example only, consider the amino acid leucine; the codon CUU is used eleven times in the **gfp** gene, but this codon corresponds to only the fourth preferred codon in human genes. The leucine codon UUA also features prominently in the jellyfish gene, and this codon is the last choice for use in the human genome. Changing the Leucine codons would thus make an appropriate starting point for preparing a **humanized gene**.

Drawing Description Text - DRTX:

From studying the information in Table 3 and Table 4, one of skill in the art would readily discern that the jellyfish **gfp** codons CTA, TTA, TCG and TCA (or CUA, UUA, UCG or GUA) should be changed to a more preferred codon. As a general guideline, those codons listed in columns 5 and 6 generally represent codons that one would prefer to change in creating a **humanized gene, the codons listed in column 4 should also often be changed in creating a humanized gene**; the codons listed in column 3 may or may not be changed, depending on the number of changes that one wishes to make in total and on the particular amino acid that is to be encoded. Those codons listed in columns 1 and 2, when occurring in the wildtype **gfp** sequence, will generally be appropriate and should not need changing, unless there is only a choice of two codons available. However, replacing a codon from column 2 with a codon from column 1 is certainly a useful option, particularly where there is only a choice of two codons. Given this information, it will now be understood that, when introducing changes into the **gfp** sequence, one would generally desire to introduce a codon of column 1 wherever possible.

Drawing Description Text - DRTX:

In light of the foregoing discussion, it will be clear that the exemplary sequence of SEQ ID NO:3 is only one of the many operable species that are encompassed by the present invention. In SEQ. ID NO:3, 88 codons contain one or more base substitutions. 88 codons from a sequence that encodes 328 amino acids represents a change of about 37%. However, it is contemplated that changing about 10% of the codons would produce a useful increase in expression levels and such gene sequences therefore fall within the scope of the present invention. Changing about 15%, 20%, 25% or 30% of the codons within the jellyfish **gfp sequence is also considered to be useful and the humanized genes** of this invention encompass those gene sequences that fall within the aforementioned ranges.

Drawing Description Text - DRTX:

In certain embodiments, depending on the nature of the codon changes introduced, it may not be necessary to even make a 10% change in the codon usage of the **gfp** gene. For example, if each of the ten least favored codons were to be changed and replaced with those most preferred for use in human genes, it is contemplated that the resultant sequence may achieve reasonable expression in human and mammalian cells. Changing ten codons from within 328 represents a percentage change of about 4%. Therefore, so-called "4% **humanized genes**" also fall within the scope of the present invention given the following provision - that when making only a limited number of changes, one would generally wish to change the ten codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the **gfp** gene sequence. When making these key changes along with a number of other changes, it is contemplated that changing at least about 7, 8 or 9 of these codons will be sufficient to result in a **humanized gene** with improved expression. As described above, leucine would preferably be encoded by CTG, CTC or TTG; valine would preferably be encoded by GTG; and serine would preferably be encoded by AGC.

Drawing Description Text - DRTX:

Although **gfp** gene sequences in which about 4-5, about 10, about 20 or about 30-35% of the codons have been changed will generally be preferred, there is no reason that further changes should not be made if so desired. **Humanized gene sequences in accordance with the present invention may therefore be sequences that contain humanized** codons at about 40%, 50%, 60%, 70% or even about 80-90% of the codon positions within the full length codon region. In reviewing SEQ ID NO:3, with a view to introducing still further humanizing changes, a number of positions are identifiable in which further optimizing changes could be introduced. These include, for example, those codons found at codon positions 6, 9, 14, 17, 19, 21, 23, 26, 27, 31, 33, 34, 35, 36, 40, 45, 50, 51, 62, 71, 83, 99, 101, 102, 111, 115, 116, 128, 130, 132, 133, 134, 136, 142, 157, 171, 173, 174, 181, 183, 186, 209, 210, 213, 223 and 230 of SEQ. ID NO:3.

Drawing Description Text - DRTX:

The humanized **GFP** of the present invention renders several of these methods practical rather than speculative. **Humanized gfp genes** can therefore be used to identify transformed cells, e.g., by fluorescence-activated cell sorting (FACS) or fluorescence microscopy; to measure gene expression in vitro and in vivo; to label specific cells in multicellular organisms, e.g., to study cell lineages; to label and locate fusion proteins; and to study intracellular trafficking and the like.

Drawing Description Text - DRTX:

In methods to produce fluorescent molecular weight markers, a **humanized gfp gene** sequence is generally fused to one or more DNA sequences that encode proteins having defined amino acid sequences and the fusion proteins are expressed from an expression vector. Expression results in the production of fluorescent proteins of defined molecular weight or weights that may be used as markers (following calculation of the size of the complete amino acid).

Drawing Description Text - DRTX:

A first example of this general group is where a **humanized gfp sequence** is fused to a DNA sequence encoding a selected protein in order to directly label the encoded protein with **GFP**. Expressing such a humanized **GFP** fusion protein in a cell results in the production of fluorescently-tagged proteins that can be readily detected. This is useful in simply confirming that a protein is being produced by a chosen host cell. It also allows the location of the selected protein to be determined, whether this represents a natural location or whether the protein has been targeted to an organelle by the hand of man.

Drawing Description Text - DRTX:

The **humanized genes** of this invention also provide another dimension to the analysis of promoters in mammalian cells. As **gfp** can now be expressed in mammalian and human cells and readily detected, a range of promoters can be tested for their suitability for use with a given gene, cell, or system. This applies to in vitro uses, such as in identifying a suitable promoter for use in recombinant expression and high level protein production, and also in in vivo uses, such as in pre-clinical testing or in gene therapy in human subjects.

Drawing Description Text - DRTX:

The use of **humanized gfp genes** with inducible promoters also extends to an analysis of the promoter itself. An example here is in the analysis of a particular promoter from a group of promoters, such as promoters associated with heat shock proteins, that are known to be expressed in various organisms throughout evolution. In this way, a promoter operable in, for example, yeast, can be taken and expressed in a mammalian cell system in order to determine whether it is operable in mammalian cells and, therefore, to determine whether mammalian cells likely include a homolog of the yeast promoter.

Drawing Description Text - DRTX:

In the screening embodiments, the **humanized gfp gene** will be positioned downstream of a promoter that is known to be inducible by the agent that one wishes to identify. Expression of **gfp** in the cells will normally be silent, and will be switched on by exposing the cell to a composition that contains the selected agent. In using a promoter that is responsive to, for example, a heavy metal, a toxin, a hormone, a cytokine or other defined molecule, the presence of a heavy metal, toxin, hormone, cytokine or such like can readily be determined.

Drawing Description Text - DRTX:

In the biological assays, cells including a **humanized gfp gene** under the control of a promoter that is inducible by a biological effector molecule may be used to detect the presence of such molecules in various kinds of biological samples, including blood, plasma, semen, urine, saliva. and the like. Those effector molecules that are detectable in this way include molecules such as hormones, cytokines, neurotransmitters and the like. Of course, as used throughout this application, it will be understood that the term "promoter" is being used to refer to any **regulatory** element. Particular examples here are the use of the sterol **regulatory** element, in conjunction with humanized **gfp**, to detect sterols in a given composition; and the similar use of the serum response element, which is induced by UV, EGF, PDGF and TPA.

Drawing Description Text - DRTX:

In the so-called chemical assays, cells including a **humanized gfp gene** under

the control of a promoter that is inducible by a chemical agent are used to detect the presence of the chemical agent in various compositions. These assays may be used to detect toxins or contaminants in fluids such as drinking water, and the like. The types of agents that may be detected in this way include heavy metals, toxins and various other pollutants and undesirable chemicals.

Drawing Description Text - DRTX:

Humanized gfp genes can be used as one portion of a fusion protein, allowing the location of the protein to be identified. Fusions of GFP with an 'exogenous' protein should preserve both the fluorescence of GFP and functions of the host protein, such as physiological functions and/or targeting functions.

Drawing Description Text - DRTX:

Adding a nuclear localization signal to a humanized gfp gene may also be used in order to enhance the fluorescence intensity of the expressed protein by confining the protein to the much smaller space of the nucleus. This is described herein in Example VII in the context of blue GFP mutants.

Drawing Description Text - DRTX:

Successful gene therapy generally requires the integration of a gene able to correct the genetic disorder into the host genome, where it would co-exist and replicate with the host DNA and be expressed at a level to compensate for the defective gene. Ideally, the disease would be cured by one or a few treatments, with no serious side effects. There have been several approaches to gene therapy proposed to date, each of which may benefit from combination with the humanized gfp of the present invention.

Drawing Description Text - DRTX:

As mentioned earlier, modification and changes may be made in the structure of GFP and still obtain a molecule having like or otherwise desirable characteristics. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of function. It is thus contemplated that various changes may be made in the sequence of humanized gfp proteins, by virtue of changing the underlying DNA, without appreciable loss of their biological utility or activity.

Drawing Description Text - DRTX:

Site-specific mutagenesis may be used to prepare further variants of humanized gfp genes. Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or

peptides, through specific **mutagenesis** of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants by introducing one or more nucleotide sequence changes into the DNA.

Drawing Description Text - DRTX:

In general, site-directed **mutagenesis** in accordance herewith is performed by first obtaining a single-stranded vector or melting apart the two strands of a double stranded vector which includes within its **sequence a DNA sequence which encodes gfp or humanized gfp**. An oligonucleotide primer bearing the desired **mutated** sequence is prepared, generally synthetically, for example by the method of Crea et al. (1978). This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as E. coli polymerase I Klenow fragment, in order to complete the synthesis of the **mutation**-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-**mutated** sequence and the second strand bears the desired **mutation**. This heteroduplex vector is then used to transform appropriate cells, such as E. coli cells, and clones are selected which include recombinant vectors bearing the **mutated** sequence arrangement.

Drawing Description Text - DRTX:

The preparation of **sequence variants of the selected humanized gfp gene** using site-directed **mutagenesis** is provided as a means of producing potentially useful **GFP** species and is not meant to be limiting as there are other ways in which sequence variants of **GFP** may be obtained. For example, recombinant vectors encoding the desired **humanized gfp gene** may be treated with **mutagenic** agents to obtain sequence variants (see, e.g., a method described by Eichenlaub, 1979) for the **mutagenesis** of plasmid DNA using hydroxylamine.

Drawing Description Text - DRTX:

A wide variety of recombinant plasmids and vectors may be engineered to express a **humanized gfp genes** and so used to deliver **GFP** to a cell.

Drawing Description Text - DRTX:

As used herein, the term "expression vector" includes any type of genetic construct containing a nucleic acid **sequence of a humanized gfp gene** in which the nucleic acid sequence is capable of being transcribed in a mammalian or human cell. The expression vectors of the invention should also direct translation into **GFP** protein, as provided by the invention itself. In addition to the **humanized gfp sequence**, expression vectors will generally include restriction enzyme cleavage sites and the other initial, terminal and intermediate DNA sequences that are usually employed in vectors to facilitate their construction and use.

Drawing Description Text - DRTX:

For long-term, high-yield production of recombinant proteins, stable expression is often preferred. Here, rather than using expression vectors that contain viral origins of replication, host cells can be transformed with vectors controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. The combined use of humanized gfp sequences and selectable markers is therefore also contemplated.

Drawing Description Text - DRTX:

A "promoter" refers to a DNA sequence recognized by the synthetic machinery of a cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. As used herein, the promoter should be operable in mammalian and human cells. The phrases "operable" and "exerting transcriptional control" mean that the promoter is in the correct location and orientation in relation to the humanized gfp nucleic acid to control RNA polymerase initiation and expression of the humanized gene.

Drawing Description Text - DRTX:

The promoter used to express the humanized GFP is not critical to the present invention. In the examples given, the human cytomegalovirus (CMV) immediate early gene promoter has been used (Thomsen et. al., 1984), which results in the constitutive, high-level expression of the foreign gene. However, the use of other viral or mammalian cellular promoters which are well-known in the art is also suitable to achieve expression of the humanized gfp gene.

Drawing Description Text - DRTX:

Furthermore, selection of a promoter that is regulated in response to specific chemical or physiological signals can permit inducible expression of the humanized gfp gene. Examples of suitable inducible promoters include the PAI-1, cytochrome P450 gene promoters, heat shock protein genes and hormone inducible gene promoters, and the fos and jun promoters inducible by ionizing radiation.

Drawing Description Text - DRTX:

IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. In this manner, multiple genes, one of which will be a humanized gfp gene, can be efficiently expressed using a single promoter/ enhancer to transcribe a single message.

Drawing Description Text - DRTX:

In that the vectors for use in these aspects are replication defective, they will typically not have an adenovirus E1 region. Thus, it will be most convenient to introduce the humanized gfp gene at the position from which the E1 coding sequences have been removed. However, the position of insertion of the humanized gene within the adenovirus sequences is not critical. The humanized transcriptional unit may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described previously by Karlsson et. al. (1986).

Drawing Description Text - DRTX:

Expression kits comprising humanized gfp genes form another aspect of the invention. Such kits will generally contain, in suitable container means, a formulation of a humanized gfp gene or a vector capable of expressing a humanized gfp gene. The gene or vector may be provided in a pharmaceutically acceptable formulation.

Drawing Description Text - DRTX:

When the components of the kit are provided in one or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. The humanized gfp gene or vector may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, eye dropper, or other such like apparatus, from which the formulation may be applied to a cell, or to an area of the body, or injected into an animal, or applied to and mixed with other components of a kit.

Drawing Description Text - DRTX:

The container means will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the humanized gfp gene or vector may be placed, preferably, suitably allocated. A second humanized gfp gene or vector construct may also be provided, wherein the kit will also generally contain a second vial or other container into which this is be placed. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent.

Drawing Description Text - DRTX:

Recombinant Host Cells The terms "engineered" and "recombinant" cells are intended to refer to a cell into which an exogenous DNA segment or gene that includes a humanized gfp gene sequence has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced exogenous DNA segment or gene. Engineered cells are thus cells having a gene or genes introduced through the

hand of man.

Drawing Description Text - DRTX:

Primary cells of all vertebrate species are considered for use with the humanized gfp genes disclosed herein, whether or not they are returned to the body of an animal. These include, by way of example only, bone marrow cells, nerve cells, lung epithelial cells and hepatocytes.

Drawing Description Text - DRTX:

Of course, it will be understood that as the present invention is well suited for use in more direct gene therapy methods, any target cell of the body can contain a humanized gfp gene as described in this invention. All such cells are considered to fall within the description of a "recombinant host cell", as used herein. This includes any cell within an animal or human subject that comprises one or more copies of a humanized gfp gene or vector, irrespective of the manner in which the cell acquires the gene, e.g., by transfection, infection and the like. Diseased cells, deficient cells and healthy cells are all encompassed within the invention in this manner.

Detailed Description Text - DETX:

Construction of Humanized GFP Gene and Vectors

Detailed Description Text - DETX:

This example describes the production of a further humanized GFP sequences encoding GFP protein variants with different properties to the wild type protein. The variants also have increased expression in mammalian and human cells.

Detailed Description Text - DETX:

Two mutants were constructed in the pBS-GFP.sub.h background by site-directed PCR.TM. mutagenesis. A first humanized mutant mirrors the protein sequence reported by Heim et al. (1995) who described a Ser65 to Thr65 substitution that increased the fluorescence yield in the context of the original jellyfish codon sequence. Reasoning that this mutation might be even more effective in the context of the humanized pTR.sub.BS -UF1 sequence, the inventors reproduced this point mutation in the PTR.sub.BS -UF1 background to produce plasmid pTR.sub.BS -UF2.

Detailed Description Text - DETX:

Construction of recombinant AAV vector plasmid pTR-UF1 containing humanized GFP

gene that encodes a wild-type GFP protein is described in Examples I-V.

Detailed Description Text - DETX:

Construction of recombinant AAV vector plasmid pTR-UF2 containing the humanized GFP gene that encodes a GFP protein variant with different properties from the wild-type GFP protein is described in this example. The, humanized GFP gene contained in plasmid pTR-UF2 has a point mutation, Ser65 to Thr65.

Detailed Description Text - DETX:

To compare the expression efficiency of the humanized gfp constructs with the original jellyfish sequence the inventors transfected 293 cells with pTR.sub.BS -UF, pTR.sub.BS -UF1, or pTR.sub.BS -UF2 plasmid DNA at various DNA concentrations. The transfected cells were then analyzed by FACS 36 hr after transfection (FIG. 3).

Detailed Description Text - DETX:

Results from these studies revealed that pTR.sub.BS -UF1 carrying the humanized gfp sequence consistently produced 5-10 times higher number of cells scored as positive for green fluorescence than the jellyfish sequence. The point mutation in pTR.sub.BS -UF2 increased the number of fluorescent cells by an additional 5-10 fold over pTR.sub.BS -UF1.

Detailed Description Text - DETX:

To determine whether the modified gfp cDNA was sufficient now to detect the marker gene at low gene copy number, the inventors isolated recombinant AAV viruses by packaging and using the three gfp constructs (UF, UF1, and UF2) and used them to transduce the gfp marker into 293 cells by virus infection. While there was almost no detectable GFP expression from a virus carrying the gfp10 cDNA (rAAV-GFP.sub.J), cells infected with a virus carrying the humanized gfp.sub.h gene (rAAV-GFP.sub.H 1, or rAAV-GFP.sub.H 2) were readily detected either visually (FIG. 4A and FIG. 4B), or by FACS analysis. FACS analysis was conducted by harvesting transfected 293 cells and analyzing on a flow cytometer (Becton-Dickinson) equipped for FITC detection at an excitation wavelength of 488 nm. At high M.O.I. (approximately 20) the ratio of infected cells, scored by FACS as fluorescent-positive, reached 70% for rAAV-GFP.sub.H 2.

Detailed Description Text - DETX:

Recombinant AAV vector plasmid pTR-UF5N was constructed as shown in FIG. 12. This plasmid was prepared as described for pTR-UF2 in Example V except that the humanized GFP gene contains the additional point mutations Phe64 to Leu64 and Ser65 to Thr65 and additionally has the SV40 Large T-antigen nuclear localization signal fused in frame 5' to the GFP gene. Use of the pTR-UF5N

vector resulted in increased fluorescence of the **GFP** compared with pTR-UF5 that did not contain the nuclear localization signal. pTR-UF5 is shown in FIG. 11. pTR-UF5 lacking a nuclear localization signal, has increased **GFP** fluorescence compared with pTR-UF2.

Detailed Description Text - DETX:

The present example describes the construction of a recombinant adenovirus shuttle plasmid and the construction of recombinant adenovirus expressing **humanized gfp gene**. This exemplifies the use of different vector systems in humanized **GFP** expression.

Claims Text - CLTX:

1. A **humanized green fluorescent protein (GFP) gene** comprising the sequence of SEQ ID NO:3 wherein TTC encoding phenylalanine at position 64 and TCT encoding serine at position 65 is replaced by CTG and ACC, respectively.

Claims Text - CLTX:

5. A **humanized GFP gene** encoding a polypeptide having the amino acid sequence of SEQ ID NO:18.

Claims Text - CLTX:

6. A **humanized GFP gene** encoding a polypeptide having the amino acid sequence of SEQ ID NO:20.

Claims Text - CLTX:

7. A **humanized GFP gene** operatively fused 5' to a nuclear targeting sequence.

Claims Text - CLTX:

8. The **humanized GFP of claim 7 wherein the nuclear targeting sequence** is SV40 large T-antigen nuclear localization signal.

Claims Text - CLTX:

9. The **humanized GFP of claim 8 wherein the GFP gene** is modified to express leucine64 in place of phenylalanine64 and threonine 65 in place of serine 65.

Claims Text - CLTX:

10. A **humanized GFP gene** of comprising the sequence of SEQ ID NO. 19 wherein said gene is modified at position 1210, 1211 and 1212 (CAT) and at 1457, 1458 and 1459.

Claims Text - CLTX:

11. The **humanized GFP of claim 10 wherein the nuclear targeting sequence** comprises nucleotides 987-1014 of SEQ ID NO. 19.

US-PAT-NO: 6001591

DOCUMENT-IDENTIFIER: US 6001591 A

TITLE: Recombinant bovine adenoviruses

DATE-ISSUED: December 14, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|------------|-------|----------|---------|
| Mittal; Suresh K. | Saskatoon | N/A | N/A | CA |
| Graham; Frank L. | Hamilton | N/A | N/A | CA |
| Prevec; Ludvik | Burlington | N/A | N/A | CA |
| Babiuk; Lorne A. | Saskatoon | N/A | N/A | CA |

APPL-NO: 08/ 845623

DATE FILED: April 25, 1997

PARENT-CASE:

This application is a division of application Ser. No. 08/164,292, filed Dec. 9, 1993, now U.S. Pat. No. 5,820,868.

US-CL-CURRENT: 435/69.1; 424/199.1 ; 435/235.1 ; 435/320.1

ABSTRACT:

The present invention provides recombinant bovine adenovirus (BAV) vectors and expression systems for introduction and expression of non-BAV sequences in mammalian cells. Methods for the preparation and use of these vectors and expression systems are also provided.

12 Claims, 58 Drawing figures

Exemplary Claim Number: 1,2

Number of Drawing Sheets: 51

----- KWIC -----

Detailed Description Text - DETX:

A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to,

procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, viral DNA, and even synthetic DNA sequences.

A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Detailed Description Text - DETX:

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a viral gene, the gene will usually be flanked by DNA that does not flank the viral gene in the genome of the source virus or virus-infected cells. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

Detailed Description Text - DETX:

Luciferase activity in BAV3-Luc-infected MDBK cells was monitored at different times post-infection by luciferase assays (FIG. 13). A low level of luciferase activity was first observed at 12 h post-infection reaching a peak at 30 h post-infection and then dropped subsequently. At 30 h post-infection, approximately 425 pg luciferase was detected in 4.times.10.sup.5 BAV3-Luc (3.1)-infected MDBK cells. In MDBK cells-infected with the wt BAV3, luciferase expression was not detected (data not shown). The kinetics of luciferase expression by BAV3-Luc (3.1) and BAV3-Luc (3.2) appears very much similar. The kinetics of luciferase expression also showed that the majority of enzyme expression in virus-infected cells seemed to occur late in infection. To determine luciferase expression in the absence of viral DNA replication, BAV3-Luc-infected MDBK cells were incubated in the presence of an inhibitor of DNA synthesis, 1-.beta.-D-arabinofuranosyl cytosine (AraC) and luciferase activity was measured in virus-infected cell extracts at various times post-infection and compared to luciferase expression obtained in the absence of AraC (FIG. 14). When the recombinant virus-infected cells were incubated in the presence of AraC, luciferase expression at 18, 24 and 30 h post-infection was approximately 20-30% of the value obtained in the absence of AraC. These results indicated that the majority of luciferase expression in MDBK cells infected with BAV3-Luc took place after the onset of viral DNA synthesis. To confirm this MDBK cells-infected with the BAV3-Luc were grown in the absence or presence of AraC, harvested at 18 h, 24 h, and 30 h post-infection, viral DNA extracted and analyzed by dot blot analysis using pSM51-Luc (see FIG. 9) as a probe (data not shown). In the presence of AraC, viral DNA synthesis was severely reduced compared to viral DNA synthesis in the absence of AraC. Western blot analysis of BAV3-Luc-infected cells Luciferase was expressed as an active enzyme as determined by luciferase assays using extracts from MDBK cells-infected with BAV3-Luc (see FIG. 13). The luciferase gene without any exogenous regulatory sequences was inserted into E3 of the BAV3 genome, therefore, there was a possibility of luciferase expression as a fusion protein

with part of an E3 protein if the luciferase gene was in the same frame, Such as, F1 and F3 which represent open reading frames (ORFs) for E3 proteins (FIG. 15) or the fusion protein may arise due to recognition of an upstream initiation codon in the luciferase ORF. T explore this possibility we sequenced the DNA at the junction of the luciferase gene and the BAV3 sequences with the help of a plasmid, pSM51-Luc and a synthetic primer design to bind luciferase coding sequences near the initiation codon (data not shown). The luciferase coding region fell in frame F2. The luciferase initiation codon was the first start codon in this frame, however, the ORF started at 84 nucleotides upstream of the luciferase start codon. To further confirm that luciferase protein is of the same molecular weight as purified firefly luciferase, unlabeled mock-infected, wt BAV3-infected or BAV3-Luc-infected MDBK cell extracts were reacted with an anti-luciferase antibody in a Western blot (FIG. 16). A 62 kDa polypeptide band was visible in the BAV3-Luc (lane 3 and 4)-infected cell extracts which were of the same molecular weight as pure firefly luciferase (lane 5). We are not sure whether a band of approximately 30 kDa which also reacted with the anti-luciferase antibody in lanes 3 and 4 represented a degraded luciferase protein.

US-PAT-NO: 5968750

DOCUMENT-IDENTIFIER: US 5968750 A

TITLE: Humanized green fluorescent protein genes and methods

DATE-ISSUED: October 19, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|-------------|-------|----------|---------|
| Zolotukhin; Sergei | Gainesville | FL | N/A | N/A |
| Muzyczka; Nicholas | Gainesville | FL | N/A | N/A |
| Hauswirth; William W. | Gainesville | FL | N/A | N/A |

APPL-NO: 09/ 169605

DATE FILED: October 9, 1998

PARENT-CASE:

This is a divisional of application Ser. No. 08/588,201 filed Jan. 18, 1996, now U.S. Pat. No. 5,874,304.

US-CL-CURRENT: 435/6; 435/366 ; 435/40.52 ; 435/7.21 ; 435/7.4

ABSTRACT:

Disclosed are **synthetic and "humanized" versions of green fluorescent protein (GFP) genes** adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such **humanized genes** are also disclosed. In addition, various methods for using the efficient expression of humanized **GFP** in mammalian cells and in animals are described.

30 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

----- KWIC -----

Abstract Text - ABTX:

Disclosed are **synthetic and "humanized" versions of green fluorescent protein (GFP) genes** adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order

to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such humanized genes are also disclosed. In addition, various methods for using the efficient expression of humanized GFP in mammalian cells and in animals are described.

Brief Summary Text - BSTX:

The present invention relates generally to the field of reporter genes and particularly provides improved green fluorescent protein (GFP) genes, constructs and methods of use. The gfp genes disclosed herein are humanized gfp genes adapted for expression in mammalian and human cells by using preferred DNA codons.

Brief Summary Text - BSTX:

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing humanized green fluorescent protein (GFP) genes adapted for expression in mammalian and human cells. The humanized gfp genes of the invention are prepared by incorporating codons preferred for use in human genes into the DNA sequence. Also provided are humanized gfp expression constructs and various methods of using the humanized genes and vectors.

Brief Summary Text - BSTX:

Accordingly, the present invention provides humanized green fluorescent protein (GFP) genes and methods of making and using such genes. As used herein the term a "humanized green fluorescent protein (GFP) gene" means a gene that has been adapted for expression in mammalian and human cells by replacing at least one, and preferably, more than one, and most preferably, a significant number, of jellyfish gfp codons with one or more codons that are more frequently used in human genes.

Brief Summary Text - BSTX:

The humanized genes of the invention are preferably cDNAs, although genomic copies are by no means excluded. The humanized genes are also preferably humanized versions adapted from the A. victoria gfp gene, although other gfp gene sources are, again, not excluded.

Brief Summary Text - BSTX:

In certain embodiments, the present invention provides humanized gfp genes that encode a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2.

Brief Summary Text - BSTX:

In other embodiments, **humanized gfp genes** will encode **GFP** variants that are generally based upon the foregoing sequence, but that have certain changes. A particular example is a **humanized gene** that encodes a **GFP** with an amino acid sequence of SEQ ID NO:2 in which Serine at position 65 has been replaced by Threonine.

Brief Summary Text - BSTX:

A further example is a **humanized gfp gene** that encodes a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2 in which Tyrosine at position 66 has been replaced by Histidine.

Brief Summary Text - BSTX:

Another example is a **humanized gfp gene** that encodes a **GFP** that has the amino acid sequence of SEQ ID NO:2 in which the chromophore sequence Phe Ser Tyr Gly Val Gln (SEQ ID NO:4) between positions 64 and 69 has been replaced by the sequence Met Gly Tyr Gly Val Leu (SEQ ID NO:5).

Brief Summary Text - BSTX:

Structural equivalents of the **humanized gfp genes** are also included within the present invention. However, **mutants** that are truncated by more than one amino acid residue at the amino terminus or more than about 10 or 15 amino acid residues from the carboxyl terminus are not generally considered to be useful in the context of producing a fluorescent protein. The encoded **GFP** should therefore be a minimum of about 222 amino acids in length, with proteins of about 238 amino acids in length generally being preferred.

Brief Summary Text - BSTX:

Humanized gfp genes wherein at least about 50% or above of the codon positions contain a humanized codon are also contemplated.

Brief Summary Text - BSTX:

Preferred **humanized gfp genes** of the invention are those genes that contain certain key changes. Examples are **genes that comprises at least seven humanized** codons from the 10 codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the jellyfish **gfp** sequence.

Brief Summary Text - BSTX:

Preferably, **humanized gfp genes will comprise at least eight, at least nine, or ten, humanized** codons from the 10 codons located at codon positions 18, 53, 93,

125, 150, 178, 195, 208, 236 and 224 of the jellyfish gfp gene sequence.

Brief Summary Text - BSTX:

Such constructs are exemplified by humanized genes that comprise any one of the humanized Leucine codons CTG, CTC or TTG at codon positions 18, 53, 125, 178, 195 and 236 of the GFP gene sequence. A further example is a humanized gfp gene that comprises the humanized Valine codon GTG at codon positions 93, 150 and 224 of the GFP gene sequence. Other examples are humanized genes that comprise the humanized Serine codon TCT at codon position 208 of the GFP gene sequence.

Brief Summary Text - BSTX:

The humanized gfp genes encompassed by this invention also include those genes that comprises an increased number of GCC or GCT Alanine-encoding codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO: 1.

Brief Summary Text - BSTX:

By the phrase "increased number of codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO: 1" is meant that the humanized sequence contain an increased number of codons encoding a particular amino acid within the GFP coding region that encodes the amino acid sequence of SEQ ID NO:2, or one of the mutants or other equivalents described herein, in comparison to those codons encoding the same amino acid that are present within the coding region of the wild type jellyfish gene sequence of SEQ ID NO:1. Thus it will be understood that the term "increased", when used in this context, does not mean the addition of one or more codons to a terminal portion of the coding region, but rather means replacement of an unfavorable codon within the coding region with a codon that is more favorable for translation in a human or mammalian cell.

Brief Summary Text - BSTX:

In light of the definition set forth above, the humanized gfp genes of the invention may also be defined as those genes that comprise an increased number of TGC Cysteine-encoding codons; an increased number of GAC Aspartate-encoding codons; an increased number of GAG Glutamate-encoding codons; an increased number of TTC Phenylalanine-encoding codons; an increased number of GGC Glycine-encoding codons; an increased number of CAC Histidine-encoding codons; an increased number of ATC Isoleucine-encoding codons; an increased number of CTG or CTC Leucine-encoding codons; an increased number of AAG Lysine-encoding codons; an increased number of AAC Asparagine-encoding codons; an increased number of CCC or CCT Proline-encoding codons; an increased number of CAG Glutamine-encoding codons; an increased number of CGC, AGG or CGG Arginine-encoding codons; an increased number of AGC or TCC Serine-encoding codons; an increased number of ACC Threonine-encoding codons; an increased

number of GTG or GTC Valine-encoding codons; and/or an increased number of TAC Tyrosine-encoding codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Brief Summary Text - BSTX:

In certain embodiments, the **humanized gfp genes** may also comprise a TGA termination codon.

Brief Summary Text - BSTX:

Humanized gfp genes may also be defined by comprising a decreased number of certain codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1. "Decreased" in this context also means that the humanized **sequence contain** a decreased number of codons encoding a particular amino acid within the GFP coding region that encodes the amino acid sequence of SEQ ID NO:2, or a mutant or **equivalent** thereof, in comparison to those codons encoding the same amino acid that are present within the coding region of the wild type jellyfish gene sequence of SEQ ID NO:1. Thus it will be understood that "decreased" does not in any way reflect the simple deletion of codons from any portion of the coding region, but again refers to replacement of a jellyfish codon with a codon that occurs more frequently in human genes.

Brief Summary Text - BSTX:

Accordingly, **humanized gfp genes** of the present invention are also be defined as those genes that comprise a decreased number of GCA Alanine-encoding codons; a decreased number of GGU Glycine-encoding codons; a decreased number of CTT, CTA or TTA Leucine-encoding codons; a decreased number of AGA Arginine-encoding codons; a decreased number of AGT, TCA or TCG Serine-encoding codons; or a decreased number of GTT or GTA Valine-encoding codons.

Brief Summary Text - BSTX:

Although not believed to be required, it is currently preferred that the **humanized gfp genes should include a Kozak consensus sequence operatively positioned upstream from the humanized gene** sequence (i.e., the gene is positioned downstream from the Kozak consensus sequence).

Brief Summary Text - BSTX:

Certain preferred **humanized gfp genes** will comprise the nucleic acid sequence of SEQ ID NO:3. However, this is by no means limiting and is just one exemplary embodiment of the present invention. Detailed directions as how to make and use many other such **humanized gfp genes** are included herein. For example, one may refer to the information in Table 2, Table 3 and Table 4 in creating any one of a number of suitable **humanized gfp genes**.

Brief Summary Text - BSTX:

Virtually any protein- or peptide-encoding DNA sequence, or combinations thereof, may be fused to a humanized gfp sequence in order to encode a fusion protein. This includes DNA sequences that encode targeting peptides, therapeutic proteins, proteins for recombinant expression, proteins to which one or more targeting peptides is attached, protein subunits and the like.

Brief Summary Text - BSTX:

Recombinant vectors and plasmids form another important aspect of the present invention. In such vectors, the humanized gfp gene is positioned under the transcriptional control of a promoter, generally a promoter operative in a mammalian or human cell. "Positioned under the transcriptional control of" means that the humanized gfp sequence is positioned downstream from and under the transcriptional control of the promoter such the promoter is capable of directing expression of the encoded GFP protein in a mammalian or human host cell upon introduction of the vector into such a cell.

Brief Summary Text - BSTX:

The recombinant vectors of the invention will thus generally comprise a humanized gfp reporter gene operatively positioned downstream from a promoter, wherein the promoter is capable of directing expression of the humanized GFP gene in a mammalian or human cell. Preferably the promoter will direct expression of GFP in an amount sufficient to allow GFP detection by detecting the green fluorescence following expression of GFP in the cell. Such promoters are thus "operative" in mammalian and human cells.

Brief Summary Text - BSTX:

Preferred vectors and plasmids will be constructed with at least one multiple cloning site. In certain embodiments, the expression vector will comprise a multiple cloning site that is operatively positioned between a promoter and a humanized gfp gene sequence. Such vectors may be used, in addition to their uses in other embodiments, to create N-terminal fusion proteins by cloning a second protein-encoding DNA segment into the multiple cloning site so that it is contiguous and in-frame with the humanized gfp sequence.

Brief Summary Text - BSTX:

In other embodiments, expression vectors may comprise a multiple cloning site that is operatively positioned downstream from the expressible humanized gfp gene sequence. These vectors are useful, in addition to their uses, in creating C-terminal fusion proteins by cloning a second protein-encoding DNA segment into the multiple cloning site so that it is contiguous and in-frame

with the humanized gfp sequence.

Brief Summary Text - BSTX:

In certain embodiments, the expression vector or plasmid may comprise a humanized GFP reporter gene that has the nucleic acid sequence of SEQ ID NO:3. An exemplary vector is the expression vector termed "pGREENLANTERN.TM."

Brief Summary Text - BSTX:

Reporter gene expression kits are also provided, which kits generally comprise, in suitable container means, at least one expression vector or plasmid that comprises a humanized GFP gene. The vector or plasmid will generally be one that is capable of expressing GFP in an amount sufficient to allow GFP detection by green fluorescence following expression in a mammalian or human cell.

Brief Summary Text - BSTX:

Recombinant host cells form another aspect of the present invention. Such host cells will generally comprise at least one copy of a humanized GFP gene. Preferred cells for expression purposes will be mammalian and human cells. However, it will be understood that other cell types are not excluded from those of the invention. Accordingly, cells such as bacterial, yeast, fungal, insect, nematode and plant cells are also possible, although such cells are not preferred for expression purposes.

Brief Summary Text - BSTX:

In certain embodiments, the recombinant host cells will preferably incorporate a humanized GFP gene in a manner effective to allow the cell to express, or to be stimulated to express, GFP, most preferably, in an amount sufficient to allow GFP detection by its fluorescence. The recombinant host cell will thus preferably include a humanized GFP gene that was introduced into the cell by means of a recombinant vector.

Brief Summary Text - BSTX:

In certain embodiments, the recombinant host cell will express the humanized GFP gene to produce the encoded GFP protein, preferably, in an amount sufficient to allow GFP detection by its fluorescence. It is contemplated that cells containing as few as about 20 copies of a humanized gfp gene will often express the GFP protein in an amount sufficient to allow GFP detection by green fluorescence. In certain embodiments, cells containing as few as about 10 copies, about 5 copies or even about 1 or 2 copies of a humanized gfp gene will also likely satisfy the desired expression criteria, especially where the humanized gfp gene is a mutant gene. In other embodiments, the recombinant

host cells may be capable of expressing a humanized gene in order to produce detectable GFP protein within a time frame of about 10 hours, and preferably within about 8 hours, and most preferably within about 6 hours or even less.

Brief Summary Text - BSTX:

Cells of primary cell lines that have been established after removing cells from a mammal and culturing the cells for a limited period of time are also included within the cells of the present invention. These cells may be engineered by the hand of man and returned to the same host animal from which they were originally recovered. Such cells that contain a humanized gfp gene fall within the scope of the invention, irrespective of their location.

Brief Summary Text - BSTX:

Naturally, recombinant cells also include those cells that are located within the body of an animal or human subject, as may have been targeted by gene therapy. These cells include all those that comprise at least one copy of a humanized gfp gene or vector, irrespective of the manner in which gene was acquired, e.g., by transfection, infection and the like.

Brief Summary Text - BSTX:

In certain particular embodiments, recombinant host cells that comprise a humanized GFP gene that comprises the nucleic acid sequence of SEQ ID NO:3 are contemplated.

Brief Summary Text - BSTX:

Many methods of using humanized gfp genes are provided by the present invention. The method of labeling or tagging a mammalian or human cell by expressing at least one humanized GFP gene in the cell is central to each of the methods. The humanized gfp gene should preferably produce GFP in an amount sufficient to allow ready detection of GFP in the cell by detecting GFP fluorescence.

Brief Summary Text - BSTX:

Methods of identifying a mammalian or human cell within a population of cells are also provided. Such methods generally first comprise expressing at least one humanized GFP gene in the cell in a manner effective to produce an amount of GFP sufficient to allow GFP detection by fluorescence. The cell is then admixed, or allowed to become naturally admixed, with a population of cells that do not express GFP, following which the cell is identified by means of identifying a GFP-fluorescent cell.

Brief Summary Text - BSTX:

The term "a GFP-fluorescent cell", as used herein, means that a cell expresses a humanized GFP gene in a manner effective to result in the production of the GFP product in an amount sufficient to allow subsequent detection of the cell by detecting green fluorescence from GFP in the cell.

Brief Summary Text - BSTX:

The invention further provides methods for identifying a mammalian or human cell that contains an exogenous DNA segment, which methods generally first comprise introducing into a mammalian or human cell an expression vector comprising a humanized GFP gene operatively linked to an exogenous DNA segment. The cell is then preferably cultured under conditions and for a period of time effective to allow expression of the humanized gfp gene in order to produce an amount of GFP sufficient to allow GFP detection by green fluorescence. Subsequently identifying a cell that contains the exogenous DNA segment is then achieved by identifying a GFP-fluorescent cell.

Brief Summary Text - BSTX:

In certain such embodiments, the expression vector for use in such methods will comprise a first coding region defined as the humanized gfp gene that encodes GFP and will also comprise a second coding region that comprises the exogenous DNA segment. These vectors are generally known as vectors that comprises at least two transcriptional or translational units. Two transcriptional units will naturally include two promoters that direct expression of their respective downstream genes.

Brief Summary Text - BSTX:

Two or more humanized gfp genes, each expressing a GFP protein with different spectral properties, may be detected in a cell in the manner described above. GFP-fluorescent cells, whether expressing one, two or more humanized gfp genes, may be identified by a variety of methods, including microscopy and fluorescence activated cell sorting (FACS).

Brief Summary Text - BSTX:

Further examples of methods of the invention are methods for determining the location of a selected protein within a mammalian or human cell. These methods generally comprise first introducing into a cell an expression vector comprising a contiguous DNA sequence comprising a humanized GFP gene operatively linked to a gene encoding said selected protein. The vector will generally express a fusion protein comprising GFP operatively linked to the selected protein, wherein the fusion protein is produced in amounts sufficient to allow cell detection by detecting the green fluorescence of GFP. One can then identify the location of the selected protein within the cell by

identifying the location of the green fluorescence from GFP.

Brief Summary Text - BSTX:

Still further examples of methods of the invention are methods for targeting a protein to a selected location within a mammalian or human cell. These methods generally comprise first introducing into the cell an expression vector comprising a DNA sequence comprising a DNA sequence element that encodes a targeting peptide operatively linked and contiguous with a DNA sequence element of a humanized GFP gene, which is also operatively linked and contiguous with a DNA sequence element that encodes a protein. Such vectors express a fusion protein comprising a targeting peptide operatively linked to GFP and to a protein, wherein the fusion protein is produced in the cell in an amount sufficient to allow cell detection by detecting the GFP fluorescence. The protein is then targeted to a selected location within the cell and the location is confirmed by identifying the location of the green fluorescence.

Brief Summary Text - BSTX:

These methods generally comprises introducing into a cell an expression vector comprising a humanized GFP gene under the control of the candidate promoter and maintaining the cell under conditions effective and for a period of time sufficient to allow expression of the humanized GFP gene by the candidate promoter. "Conditions effective" and "periods of time sufficient" are defined as those conditions and times that would ordinarily result in GFP being produced in an amount sufficient to allow GFP detection by green fluorescence when using a known operative promoter.

Brief Summary Text - BSTX:

A further example of methods for using humanized gfp in the context of promoters are those methods for detecting substances that stimulate transcription from a selected promoter in a mammalian or human cell. Again, one generally introduces into a mammalian or human cell an expression vector comprising a humanized GFP gene under the control of a given promoter. One then exposes the cell to a composition suspected of containing a substance known or suspected to be capable of stimulating transcription from the given promoter. The cell is then cultured or maintained for a period of time that would ordinarily allow an active promoter to stimulate GFP-fusion protein production in an amount sufficient to allow cell detection by detecting the GFP-derived green fluorescence. The subsequent identification of a GFP-fluorescent cell is then indicative of the original presence of a substance that stimulates transcription from the given promoter.

Brief Summary Text - BSTX:

These methods are also suitable for use in vitro and in vivo. In vitro uses allow substances such as toxins and pollutants to be detected by using appropriate

promoters within the humanized gfp gene constructs.

Brief Summary Text - BSTX:

As part of gene therapy, it is often necessary to determine gene expression levels in the treated mammalian animal or human subject. The present invention also provides methods for determining such the expression levels. These methods generally comprise expressing in cells of the animal an expression vector comprising a humanized GFP gene operatively linked to a selected gene. The expression vector will preferably be either a vector that expresses a GFP-fusion protein or a vector in which the humanized gfp gene and the selected protein gene each use the same or an equivalent promoter. The promoter will have preferably been shown to result in sufficient GFP expression to allow detection in vitro. One then determines the GFP-fluorescence in the cells of the animal, wherein the level of GFP-fluorescence is indicative of the expression level of the selected gene in the animal.

Brief Summary Text - BSTX:

These methods can be adapted to provide methods for analyzing the expression of a selected gene in different tissues of a mammal or human subject. Such methods generally comprise introducing into the cells of the mammal an expression vector comprising the selected gene under the control of the natural gene promoter, wherein the gene is operatively linked to a humanized GFP gene. The vector will preferably express a fusion protein that comprises the encoded gene product operatively linked to GFP, the fusion protein being produced in an amount sufficient to allow cell detection by detecting the green fluorescence of GFP. After maintaining the mammal under conditions effective and for a period of time sufficient to allow expression of the gene one then analyzes the cells of the tissues of the mammal to detect GFP-fluorescent cells, wherein the presence of GFP-fluorescent cells in a given tissue is indicative of gene expression in the tissue.

Brief Summary Text - BSTX:

A further example in which the humanized gfp genes may be employed is in the recombinant production of GFP itself. Such methods of using a humanized GFP gene simply comprise expressing the humanized gene in a mammalian or human host cell and collecting the GFP expressed by said cell.

Brief Summary Text - BSTX:

(a) preparing a recombinant vector in which a humanized GFP gene is positioned under the control of a promoter operative in a mammalian or human cell;

Brief Summary Text - BSTX:

Adaptations of such methods include those wherein the **humanized GFP gene** is fused to a DNA sequence encoding a protein or peptide of known molecular weight. Expression by the host cell thus results in a **GFP** fusion protein that may be used as a fluorescent molecular weight marker. A range of such fluorescent molecular weight markers could be so-produced to produce a molecular weight determining kit.

Drawing Description Text - DRTX:

FIG. 1. Nucleotide sequence of the gfp10 cDNA and the deduced amino acid sequence. Above each codon is the single letter designation for the amino acid. The **mutations** introduced in the gfp.sub.h sequence are shown below the substituted nucleotide of gfp10. The horizontal lines underline overlap regions of mutually priming oligonucleotides used to synthesize the gfp.sub.h cDNA. The sites of the restriction enzymes used to assemble extended pairs of oligonucleotides are shown in bold letters. The codons **mutated** to produce the Ser.sub.65 Thr **mutation**, which produces higher fluorescence yield, and the Tyr.sub.66 His **mutation**, which produces blue fluorescence, are shown in bold. In FIG. 1, the jellyfish gfp10 nucleotide sequence is SEQ ID NO:1. The deduced amino acid sequence is SEQ ID NO:2. In SEQ ID NO:2, Xaa at position 65 may be Ser or Thr; and Xaa at position 66 may be Tyr or His. The exemplary **humanized gfp sequence** shown below the substituted nucleotide of gfp10 in FIG. 1 is SEQ ID NO:3. In SEQ ID NO:3, the nucleotides at positions 193, 195 and 196 may be changed in order to encode either Ser or Thr; and either Tyr or His, as above.

Detailed Description Text - DETX:

For fluorescence microscopy, the inventors increased the sensitivity of the **GFP** reporter **gene system approximately 22 fold for one humanized** construct and at least 45 fold for a second humanized construct. In FACS analyses with **humanized gene** constructs, one construct was at least 32-fold more detectable than the original jellyfish gene, and the other construct was 190-fold more detectable than the original jellyfish gene. When humanized **GFP** is stably integrated as part of the **gfp**-neo cassette of the rAAV provirus in G418-resistant cell lines, a considerable portion of the cells express a visually detectable **GFP**.

Detailed Description Text - DETX:

In addition, the inventors describe the construction of an Ad shuttle vector, carrying the **humanized GFP reporter gene** under the control of the IRES element. 293 cells infected with recombinant Ad displayed typical CPE and bright green fluorescence. Expression of the **GFP** allowed for quick and easy selection of true recombinant Ad clones, discriminating them from false plaques.

Detailed Description Text - DETX:

The humanized **GFP** can also be incorporated into other viral and non-viral

vector and expression systems. Using the humanized genes and vectors of the present invention, efficient transduction and expression of gfp gene sequences in mammalian and human cell lines is possible. This is exemplified by gene expression in vivo within neurosensory cells of guinea pig eye, shown herein. The humanized gfp genes have many uses, such as in cell sorting by fluorescence-activated cell sorting (FACS), and in human gene therapy.

Detailed Description Text - DETX:

Green fluorescent protein genes and functional proteins are believed to be present in a variety of organisms, as shown in Table 1. A gfp gene from any of the bioluminescent cnidaria and ctenophora that express such genes can be used as the starting point for preparing a humanized gfp gene in accordance with the present invention.

Detailed Description Text - DETX:

It is currently preferred that the gfp gene sequence from A. victoria be used as the template for creating a humanized g gene, as this is readily available.

Detailed Description Text - DETX:

4. Humanized gfp Genes

Detailed Description Text - DETX:

The foregoing emphasizes the importance of the present invention, the focus of which is to provide for increased GFP expression in mammalian and human cells. Each of the mutants described above, or indeed any desired mutant or a panel of mutants, can also be prepared in a humanized background as provided by the present invention. This is because the humanizing aspects of the invention change the DNA sequence independently of the protein sequence.

Detailed Description Text - DETX:

The approach taken by the present inventors is in contrast to the Adams et. al. (1995) method, and addresses the poor translation efficiency of GFP mRNA in the human cell environment by using cDNAs that contain base substitutions in order to change the codon usage so that it is more appropriate for expression in mammalian cells. Using such humanized constructs results in green fluorescence in cells that have a low copy number of humanized gfp genes, e.g., in the range of less than 10, and even about 1 or 2 when using certain humanized gfp mutant genes.

Detailed Description Text - DETX:

The correlation between the abundance of tRNAs and the occurrence of the respective codons in protein-expressing genes has been described for *E. coli*, yeast and other organisms (Bennetzen and Hall (1982); Grantham et al. (1980); Grantham et al. (1981); Ikemura (1981a; 1981b; 1982); Wada et al. (1990)). However, until codon changes are actually made in any given gene, their effects on translation efficiency and overall expression levels cannot be established. This is similar to the situation involving the Kozak sequence, which is not believed to have been particularly helpful in increasing expression of **gfp** in mammalian cells despite expectations. Now that the present inventors have shown that **humanization is effective for gfp gene** expression, the usefulness of the **GFP** technology has been significantly enhanced.

Detailed Description Text - DETX:

An exemplary **humanized sequence** in accordance with the present invention is represented by SEQ ID NO:3. However, it will be understood that the **humanized sequences** of the present invention are by no means limited to the representative sequence of SEQ ID NO:3. Rather, in light of the following instructions, one of skill in the art will readily be able to prepare a number of different **humanized gfp sequences**.

Detailed Description Text - DETX:

Although any changes that replace a rarely used jellyfish codon with a codon that is more frequently used in human genes are considered to be useful changes, certain codon changes will naturally be preferred over others. In this regard, the inventors have identified a number of **gfp** codons that are rarely or almost never used in human genes. As discussed below, such codons are the first candidates that should be changed in producing a **humanized gene** in accordance with the present invention.

Detailed Description Text - DETX:

In making general humanizing changes, codons to be humanized can be identified by those of skill in the art from studying the information presented herein in Tables 2 and in Table 3 and 4. For example, in utilizing the information in Table 2, one would compare the frequency of the jellyfish codon against the frequency of those codons commonly used in human genes, and make any appropriate changes. By way of an example only, consider the amino acid leucine; the codon CUU is used eleven times in the **gfp** gene, but this codon corresponds to only the fourth preferred codon in human genes. The leucine codon UUA also features prominently in the jellyfish gene, and this codon is the last choice for use in the human genome. Changing the Leucine codons would thus make an appropriate starting point for preparing a **humanized gene**.

Detailed Description Text - DETX:

From studying the information in Table 3 and Table 4, one of skill in the art

would readily discern that the jellyfish **gfp** codons CTA, TTA, TCG and TCA (or CUA, UUA, UCG or GUA) should be changed to a more preferred codon. As a general guideline, those codons listed in columns 5 and 6 generally represent codons that one would prefer to change in creating a **humanized gene; the codons listed in column 4 should also often be changed in creating a humanized gene;** the codons listed in column 3 may or may not be changed, depending on the number of changes that one wishes to make in total and on the particular amino acid that is to be encoded. Those codons listed in columns 1 and 2, when occurring in the wildtype **gfp** sequence, will generally be appropriate and should not need changing, unless there is only a choice of two codons available. However, replacing a codon from column 2 with a codon from column 1 is certainly a useful option, particularly where there is only a choice of two codons. Given this information, it will now be understood that, when introducing changes into the **gfp** sequence, one would generally desire to introduce a codon of column 1 wherever possible.

Detailed Description Text - DETX:

In light of the foregoing discussion, it will be clear that the exemplary sequence of SEQ ID NO:3 is only one of the many operable species that are encompassed by the present invention. In SEQ. ID NO:3, 88 codons contain one or more base substitutions. 88 codons from a sequence that encodes 328 amino acids represents a change of about 37%. However, it is contemplated that changing about 10% of the codons would produce a useful increase in expression levels and such gene sequences therefore fall within the scope of the present invention. Changing about 15%, 20%, 25% or 30% of the codons within the **jellyfish gfp sequence is also considered to be useful and the humanized genes** of this invention encompass those gene sequences that fall within the aforementioned ranges.

Detailed Description Text - DETX:

In certain embodiments, depending on the nature of the codon changes introduced, it may not be necessary to even make a 10% change in the codon usage of the **gfp** gene. For example, if each of the ten least favored codons were to be changed and replaced with those most preferred for use in human genes, it is contemplated that the resultant sequence may achieve reasonable expression in human and mammalian cells. Changing ten codons from within 328 represents a percentage change of about 4%. Therefore, so-called "4% **humanized genes**" also fall within the scope of the present invention given the following provision--that when making only a limited number of changes, one would generally wish to change the ten codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the **gfp** gene sequence. When making these key changes along with a number of other changes, it is contemplated that changing at least about 7, 8 or 9 of these codons will be sufficient to result in a **humanized gene** with improved expression. As described above, leucine would preferably be encoded by CTG, CTC or TTG; valine would preferably be encoded by GTG; and serine would preferably be encoded by AGC.

Detailed Description Text - DETX:

Although **gfp** gene sequences in which about 4-5, about 10, about 20 or about 30-35% of the codons have been changed will generally be preferred, there is no reason that further changes should not be made if so desired. **Humanized gene sequences in accordance with the present invention may therefore be sequences that contain humanized** codons at about 40%, 50%, 60%, 70% or even about 80-90% of the codon positions within the full length codon region. In reviewing SEQ ID NO:3, with a view to introducing still further humanizing changes, a number of positions are identifiable in which further optimizing changes could be introduced. These include, for example, those codons found at codon positions 6, 9, 14, 17, 19, 21, 23, 26, 27, 31, 33, 34, 35, 36, 40, 45, 50, 51, 62, 71, 83, 99, 101, 102, 111, 115, 116, 128, 130, 132, 133, 134, 136, 142, 157, 171, 173, 174, 181, 183, 186, 209, 210, 213, 223 and 230 of SEQ. ID NO:3.

Detailed Description Text - DETX:

The humanized **GFP** of the present invention renders several of these methods practical rather than speculative. **Humanized gfp genes** can therefore be used to identify transformed cells, e.g., by fluorescence-activated cell sorting (FACS) or fluorescence microscopy; to measure gene expression in vitro and in vivo; to label specific cells in multicellular organisms, e.g., to study cell lineages; to label and locate fusion proteins; and to study intracellular trafficking and the like.

Detailed Description Text - DETX:

In methods to produce fluorescent molecular weight markers, a **humanized gfp gene** sequence is generally fused to one or more DNA sequences that encode proteins having defined amino acid sequences and the fusion proteins are expressed from an expression vector. Expression results in the production of fluorescent proteins of defined molecular weight or weights that may be used as markers (following calculation of the size of the complete amino acid).

Detailed Description Text - DETX:

A first example of this general group is where a **humanized gfp sequence** is fused to a DNA sequence encoding a selected protein in order to directly label the encoded protein with **GFP**. Expressing such a humanized **GFP** fusion protein in a cell results in the production of fluorescently-tagged proteins that can be readily detected. This is useful in simply confirming that a protein is being produced by a chosen host cell. It also allows the location of the selected protein to be determined, whether this represents a natural location or whether the protein has been targeted to an organelle by the hand of man.

Detailed Description Text - DETX:

The **humanized genes** of this invention also provide another dimension to the

analysis of promoters in mammalian cells. As gfp can now be expressed in mammalian and human cells and readily detected, a range of promoters can be tested for their suitability for use with a given gene, cell, or system. This applies to in vitro uses, such as in identifying a suitable promoter for use in recombinant expression and high level protein production, and also in in vivo uses, such as in pre-clinical testing or in gene therapy in human subjects.

Detailed Description Text - DETX:

The use of humanized gfp genes with inducible promoters also extends to an analysis of the promoter itself. An example here is in the analysis of a particular promoter from a group of promoters, such as promoters associated with heat shock proteins, that are known to be expressed in various organisms throughout evolution. In this way, a promoter operable in, for example, yeast, can be taken and expressed in a mammalian cell system in order to determine whether it is operable in mammalian cells and, therefore, to determine whether mammalian cells likely include a homolog of the yeast promoter.

Detailed Description Text - DETX:

In the screening embodiments, the humanized gfp gene will be positioned downstream of a promoter that is known to be inducible by the agent that one wishes to identify. Expression of gfp in the cells will normally be silent, and will be switched on by exposing the cell to a composition that contains the selected agent. In using a promoter that is responsive to, for example, a heavy metal, a toxin, a hormone, a cytokine or other defined molecule, the presence of a heavy metal, toxin, hormone, cytokine or such like can readily be determined.

Detailed Description Text - DETX:

In the biological assays, cells including a humanized gfp gene under the control of a promoter that is inducible by a biological effector molecule may be used to detect the presence of such molecules in various kinds of biological samples, including blood, plasma, semen, urine, saliva and the like. Those effector molecules that are detectable in this way include molecules such as hormones, cytokines, neurotransmitters and the like. Of course, as used throughout this application, it will be understood that the term "promoter" is being used to refer to any regulatory element. Particular examples here are the use of the sterol regulatory element, in conjunction with humanized gfp, to detect sterols in a given composition; and the similar use of the serum response element, which is induced by UV, EGF, PDGF and TPA.

Detailed Description Text - DETX:

In the so-called chemical assays, cells including a humanized gfp gene under the control of a promoter that is inducible by a chemical agent are used to detect the presence of the chemical agent in various compositions. These assays may be used to detect toxins or contaminants in fluids such as drinking

water, and the like. The types of agents that may be detected in this way include heavy metals, toxins and various other pollutants and undesirable chemicals.

Detailed Description Text - DETX:

Humanized gfp genes can be used as one portion of a fusion protein, allowing the location of the protein to be identified. Fusions of GFP with an 'exogenous' protein should preserve both the fluorescence of GFP and functions of the host protein, such as physiological functions and/or targeting functions.

Detailed Description Text - DETX:

Adding a nuclear localization signal to a humanized gfp gene may also be used in order to enhance the fluorescence intensity of the expressed protein by confining the protein to the much smaller space of the nucleus. This is described herein in Example VII in the context of blue GFP mutants.

Detailed Description Text - DETX:

Successful gene therapy generally requires the integration of a gene able to correct the genetic disorder into the host genome, where it would co-exist and replicate with the host DNA and be expressed at a level to compensate for the defective gene. Ideally, the disease would be cured by one or a few treatments, with no serious side effects. There have been several approaches to gene therapy proposed to date, each of which may benefit from combination with the humanized gfp of the present invention.

Detailed Description Text - DETX:

As mentioned earlier, modification and changes may be made in the structure of GFP and still obtain a molecule having like or otherwise desirable characteristics. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of function. It is thus contemplated that various changes may be made in the sequence of humanized gfp proteins, by virtue of changing the underlying DNA, without appreciable loss of their biological utility or activity.

Detailed Description Text - DETX:

Site-specific mutagenesis may be used to prepare further variants of humanized gfp genes. Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants by introducing one or more nucleotide sequence changes into the DNA.

Detailed Description Text - DETX:

In general, site-directed **mutagenesis** in accordance herewith is performed by first obtaining a single-stranded vector or melting apart the two strands of a double stranded vector which includes within its **sequence a DNA sequence which encodes gfp or humanized gfp**. An oligonucleotide primer bearing the desired **mutated** sequence is prepared, generally synthetically, for example by the method of Crea et al. (1978). This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as E. coli polymerase I Klenow fragment, in order to complete the synthesis of the **mutation**-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-**mutated** sequence and the second strand bears the desired **mutation**. This heteroduplex vector is then used to transform appropriate cells, such as E. coli cells, and clones are selected which include recombinant vectors bearing the **mutated** sequence arrangement.

Detailed Description Text - DETX:

The preparation of **sequence variants of the selected humanized gfp gene** using site-directed **mutagenesis** is provided as a means of producing potentially useful **GFP** species and is not meant to be limiting as there are other ways in which sequence variants of **GFP** may be obtained. For example, recombinant vectors encoding the desired **humanized gfp gene** may be treated with **mutagenic** agents to obtain sequence variants (see, e.g., a method described by Eichenlaub, 1979) for the **mutagenesis** of plasmid DNA using hydroxylamine.

Detailed Description Text - DETX:

A wide variety of recombinant plasmids and vectors may be engineered to expresses a **humanized gfp genes** and so used to deliver **GFP** to a cell.

Detailed Description Text - DETX:

As used herein, the term "expression vector" includes any type of genetic construct containing a nucleic acid **sequence of a humanized gfp gene** in which the nucleic acid sequence is capable of being transcribed in a mammalian or human cell. The expression vectors of the invention should also direct translation into **GFP** protein, as provided by the invention itself. In addition to the **humanized gfp sequence**, expression vectors will generally include restriction enzyme cleavage sites and the other initial, terminal and intermediate DNA sequences that are usually employed in vectors to facilitate their construction and use.

Detailed Description Text - DETX:

For long-term, high-yield production of recombinant proteins, stable expression

is often preferred. Here, rather than using expression vectors that contain viral origins of replication, host cells can be transformed with vectors controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. The combined use of humanized gfp sequences and selectable markers is therefore also contemplated.

Detailed Description Text - DETX:

A "promoter" refers to a DNA sequence recognized by the synthetic machinery of a cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. As used herein, the promoter should be operable in mammalian and human cells. The phrases "operable" and "exerting transcriptional control" mean that the promoter is in the correct location and orientation in relation to the humanized gfp nucleic acid to control RNA polymerase initiation and expression of the humanized gene.

Detailed Description Text - DETX:

The promoter used to express the humanized GFP is not critical to the present invention. In the examples given, the human cytomegalovirus (CMV) immediate early gene promoter has been used (Thomsen et. al., 1984), which results in the constitutive, high-level expression of the foreign gene. However, the use of other viral or mammalian cellular promoters which are well-known in the art is also suitable to achieve expression of the humanized gfp gene.

Detailed Description Text - DETX:

Furthermore, selection of a promoter that is regulated in response to specific chemical or physiological signals can permit inducible expression of the humanized gfp gene. Examples of suitable inducible promoters include the PAI-1, cytochrome P450 gene promoters, heat shock protein genes and hormone inducible gene promoters, and the fos and jun promoters inducible by ionizing radiation.

Detailed Description Text - DETX:

IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. In this manner, multiple genes, one of which will be a humanized gfp gene, can be efficiently expressed using a single promoter/enhancer to transcribe a single message.

Detailed Description Text - DETX:

In that the vectors for use in these aspects are replication defective, they

will typically not have an adenovirus E1 region. Thus, it will be most convenient to introduce the humanized gfp gene at the position from which the E1 coding sequences have been removed. However, the position of insertion of the humanized gene within the adenovirus sequences is not critical. The humanized transcriptional unit may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described previously by Karlsson et. al. (1986).

Detailed Description Text - DETX:

Expression kits comprising humanized gfp genes form another aspect of the invention. Such kits will generally contain, in suitable container means, a formulation of a humanized gfp gene or a vector capable of expressing a humanized gfp gene. The gene or vector may be provided in a pharmaceutically acceptable formulation.

Detailed Description Text - DETX:

When the components of the kit are provided in one or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. The humanized gfp gene or vector may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, eye dropper, or other such like apparatus, from which the formulation may be applied to a cell, or to an area of the body, or injected into an animal, or applied to and mixed with other components of a kit.

Detailed Description Text - DETX:

The container means will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the humanized gfp gene or vector may be placed, preferably, suitably allocated. A second humanized gfp gene or vector construct may also be provided, wherein the kit will also generally contain a second vial or other container into which this is be placed. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent.

Detailed Description Text - DETX:

The terms "engineered" and "recombinant" cells are intended to refer to a cell into which an exogenous DNA segment or gene that includes a humanized gfp gene sequence has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced exogenous DNA segment or gene. Engineered cells are thus cells having a gene or genes introduced through the hand of man.

Detailed Description Text - DETX:

Primary cells of all vertebrate species are considered for use with the humanized gfp genes disclosed herein, whether or not they are returned to the body of an animal. These include, by way of example only, bone marrow cells, nerve cells, lung epithelial cells and hepatocytes.

Detailed Description Text - DETX:

Of course, it will be understood that as the present invention is well suited for use in more direct gene therapy methods, any target cell of the body can contain a humanized gfp gene as described in this invention. All such cells are considered to fall within the description of a "recombinant host cell", as used herein. This includes any cell within an animal or human subject that comprises one or more copies of a humanized gfp gene or vector, irrespective of the manner in which the cell acquires the gene, e.g., by transfection, infection and the like. Diseased cells, deficient cells and healthy cells are all encompassed within the invention in this manner.

Detailed Description Text - DETX:

Construction of Humanized GFP Gene and Vectors

Detailed Description Text - DETX:

This example describes the production of a further humanized GFP sequences encoding GFP protein variants with different properties to the wild type protein. The variants also have increased expression in mammalian and human cells.

Detailed Description Text - DETX:

Two mutants were constructed in the pBS-GFP.sub.h background by site-directed PCR.TM. mutagenesis. A first humanized mutant mirrors the protein sequence reported by Heim et al. (1995) who described a Ser65 to Thr65 substitution that increased the fluorescence yield in the context of the original jellyfish codon sequence. Reasoning that this mutation might be even more effective in the context of the humanized pTR.sub.BS -UF1 sequence, the inventors reproduced this point mutation in the pTR.sub.BS -UF1 background to produce plasmid pTR.sub.BS -UF2.

Detailed Description Text - DETX:

To compare the expression efficiency of the humanized gfp constructs with the original jellyfish sequence the inventors transfected 293 cells with pTR.sub.BS -UF, pTR.sub.BS -UF1, or pTR.sub.BS -UF2 plasmid DNA at various DNA concentrations. The transfected cells were then analyzed by FACS 36 hr after transfection (FIG. 3).

Detailed Description Text - DETX:

Results from these studies revealed that pTR.sub.BS -UF1 carrying the humanized gfp sequence consistently produced 5-10 times higher number of cells scored as positive for green fluorescence than the jellyfish sequence. The point mutation in pTR.sub.BS -UF2 increased the number of fluorescent cells by an additional 5-10 fold over pTR.sub.BS -UF1.

Detailed Description Text - DETX:

To determine whether the modified gfp cDNA was sufficient now to detect the marker gene at low gene copy number, the inventors isolated recombinant AAV viruses by packaging and using the three gfp constructs (UF, UF1, and UF2) and used them to transduce the gfp marker into 293 cells by virus infection. While there was almost no detectable GFP expression from a virus carrying the gfp10 cDNA (rAAV-GFP.sub.J), cells infected with a virus carrying the humanized gfp.sub.h gene (rAAV-GFP.sub.H 1, or rAAV-GFP.sub.H 2) were readily detected either visually (FIG. 4A and FIG. 4B), or by FACS analysis. FACS analysis was conducted by harvesting transfected 293 cells and analyzing on a flow cytometer (Becton-Dickinson) equipped for FITC detection at an excitation wavelength of 488 nm. At high M.O.I. (approximately 20) the ratio of infected cells, scored by FACS as fluorescent-positive, reached 70% for rAAV-GFP.sub.H 2.

Detailed Description Text - DETX:

The present example describes the construction of a recombinant adenovirus shuttle plasmid and the construction of recombinant adenovirus expressing humanized gfp gene. This exemplifies the use of different vector systems in humanized GFP expression.

Claims Text - CLTX:

1. A method of labeling a mammalian cell, comprising expressing a humanized GFP gene in said cell.

Claims Text - CLTX:

(a) expressing a humanized GFP gene in said cell;

Claims Text - CLTX:

(a) introducing into said cell an expression vector comprising a humanized GFP gene operatively linked to an exogenous DNA segment; and

Claims Text - CLTX:

12. The method of claim 3, wherein said cell comprises a first and second **humanized GFP gene**, each expressing a **GFP** protein with different spectral properties.

Claims Text - CLTX:

(a) introducing into said cell an expression vector comprising a contiguous DNA **sequence comprising a humanized GFP gene** operatively linked to a gene encoding said selected protein; and

Claims Text - CLTX:

(a) introducing into said cell an expression vector comprising a contiguous DNA **sequence comprising a sequence encoding a targeting peptide operatively linked to humanized GFP gene** and protein-encoding gene; and

Claims Text - CLTX:

(a) introducing into said cell an expression vector comprising a **humanized GFP gene** under the control of said candidate promoter;

Claims Text - CLTX:

(b) maintaining said cell under conditions effective and for a period of time sufficient to allow expression of said **humanized GFP gene** by said candidate promoter; and

Claims Text - CLTX:

(a) introducing into a mammalian cell an expression vector comprising a **humanized GFP gene** under the control of said selected promoter;

Claims Text - CLTX:

(a) expressing in the cells of said mammal an expression vector comprising a **humanized GFP gene** operatively linked to a selected gene; and

Claims Text - CLTX:

(a) introducing into the cells of said mammal an expression vector comprising said selected gene under the control of the natural **gene promoter, said gene operatively linked to a humanized GFP gene**;

Claims Text - CLTX:

29. A method of using a **humanized GFP gene, comprising expressing a humanized GFP gene** in a mammalian host cell and collecting the **GFP** expressed by said cell.

Claims Text - CLTX:

30. The method of claim 29, wherein said **humanized GFP gene** is fused to a DNA sequence encoding a protein or peptide of known molecular weight and wherein said host cell expresses a **GFP** fusion protein.

US-PAT-NO: 5891994

DOCUMENT-IDENTIFIER: US 5891994 A

TITLE: Methods and compositions for impairing multiplication of HIV-1

DATE-ISSUED: April 6, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-------------|-------|----------|---------|
| Goldstein; Gideon | Short Hills | NJ | N/A | N/A |

APPL-NO: 08/ 893853

DATE FILED: July 11, 1997

US-CL-CURRENT: 530/329; 424/184.1 ; 424/188.1 ; 424/204.1 ; 424/208.1
; 530/324 ; 530/325 ; 530/326 ; 530/327 ; 530/328 ; 530/350

ABSTRACT:

A composition is provided which contains a non-naturally occurring peptide or polypeptide comprising at least two or more, and preferably all four amino acid sequences -Asp-Pro-Arg-Leu-Glu-Pro- SEQ ID NO: 6; -Asp-Pro-Lys-Leu-Glu-Pro- SEQ ID NO: 7; -Asp-Pro-Ser-Leu-Glu-Pro- SEQ ID NO: 8; and -Asp-Pro-Asn-Leu-Glu-Pro- SEQ ID NO: 9. This composition, including a number of additional optional peptides or polypeptides, and in a variety of forms, demonstrates a biological activity of inducing antibodies that react with most HIV-1 Tat proteins and impairing the multiplication of HIV-1. Also provided are synthetic genes encoding these peptides, recombinant viruses and commensal bacterium carrying these genes, transfected host cells, and polyclonal or other types of antibodies produced by immunizing other mammals with these aforementioned compositions. Methods for making and using such compositions to lower the viral level of HIV-1 are described.

35 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

----- KWIC -----

Brief Summary Text - BSTX:

In yet a further aspect, the invention provides a **synthetic molecule comprising the above-described synthetic gene**, operatively linked to **regulatory** nucleic acid **sequences, which direct and control expression of the product of the**

synthetic gene in a host cell.

Detailed Description Text - DETX:

The **synthetic gene of the present invention may also be part of a synthetic** or recombinant molecule. The synthetic molecule may be a nucleic acid construct, such as a vector or plasmid which contains the **synthetic gene** encoding the protein, peptide, polypeptide, fusion protein or fusion peptide under the operative control of nucleic acid sequences encoding **regulatory** elements such as promoters, termination signals, and the like. Such synthetic molecules may be used to produce the polypeptide/peptide immunogen compositions recombinantly.

Detailed Description Text - DETX:

Combinations of these techniques may be used, such as for production of the **synthetic gene**, which may require assembly of sequential immunogens by conventional molecular biology techniques, and site-directed **mutagenesis** to provide desired sequences of immunogens. The product of the **synthetic gene** is then produced recombinantly. All of these manipulations may be performed by conventional methodology.

Detailed Description Text - DETX:

Briefly, the DNA encoding the selected peptide/polypeptide is inserted into a vector or plasmid which contains other optional flanking sequences, a promoter, an mRNA leader sequence, an initiation site and other **regulatory** sequences capable of directing the multiplication and expression of that sequence in vivo or in vitro. These vectors permit infection of patient's cells and expression of the **synthetic gene** sequence in vivo or expression of it as a protein/peptide or fusion protein/peptide in vitro.

Detailed Description Text - DETX:

These data demonstrate that both epitope 1 and epitope 2 **sequences are expressed in the linear fusion protein, and react with antibodies to the synthetic sequences** (see above). Antibodies to epitope 1 were detectably induced by the recombinant fusion protein under the conditions of this experiment in mice. Thus recombinant linear expression is effective for induction of specific antibodies to epitope 1. The apparent failure of this experiment to induce antibodies to epitope 2 sequences is believed to be probably due to low immunogenicity in mice or some other experimental factor. It is anticipated that additional experiments with a more immunogenic fusion partner than **GFP** will demonstrate that the epitope 2 sequences also induce antibody responses, and thus are useful components of the compositions of this invention.

US-PAT-NO: 5874304

DOCUMENT-IDENTIFIER: US 5874304 A

TITLE: Humanized green fluorescent protein genes and methods

DATE-ISSUED: February 23, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|-------------|-------|----------|---------|
| Zolotukhin; Sergei | Gainesville | FL | N/A | N/A |
| Muzyczka; Nicholas | Gainesville | FL | N/A | N/A |
| Hauswirth; William W. | Gainesville | FL | N/A | N/A |

APPL-NO: 08/ 588201

DATE FILED: January 18, 1996

US-CL-CURRENT: 435/366; 435/320.1 ; 435/325 ; 435/354 ; 435/357 ; 435/358 ; 435/365 ; 435/367 ; 536/23.1 ; 536/23.5

ABSTRACT:

Disclosed are **synthetic and "humanized" versions of green fluorescent protein (GFP) genes** adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such **humanized genes** are also disclosed. In addition, various methods for using the efficient expression of humanized **GFP** in mammalian cells and in animals are described.

81 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

----- KWIC -----

Abstract Text - ABTX:

Disclosed are **synthetic and "humanized" versions of green fluorescent protein (GFP) genes** adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such **humanized genes** are also disclosed. In addition, various methods for using the efficient expression of humanized

GFP in mammalian cells and in animals are described.

Brief Summary Text - BSTX:

The present invention relates generally to the field of reporter genes and particularly provides improved green fluorescent protein (GFP) genes, constructs and methods of use. The gfp genes disclosed herein are humanized gfp genes adapted for expression in mammalian and human cells by using preferred DNA codons.

Brief Summary Text - BSTX:

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing humanized green fluorescent protein (GFP) genes adapted for expression in mammalian and human cells. The humanized gfp genes of the invention are prepared by incorporating codons preferred for use in human genes into the DNA sequence. Also provided are humanized gfp expression constructs and various methods of using the humanized genes and vectors.

Brief Summary Text - BSTX:

Accordingly, the present invention provides humanized green fluorescent protein (GFP) genes and methods of making and using such genes. As used herein the term a "humanized green fluorescent protein (GFP) gene" means a gene that has been adapted for expression in mammalian and human cells by replacing at least one, and preferably, more than one, and most preferably, a significant number, of jellyfish gfp codons with one or more codons that are more frequently used in human genes.

Brief Summary Text - BSTX:

The humanized genes of the invention are preferably cDNAs, although genomic copies are by no means excluded. The humanized genes are also preferably humanized versions adapted from the A. Victoria gfp gene, although other gfp gene sources are, again, not excluded.

Brief Summary Text - BSTX:

In certain embodiments, the present invention provides humanized gfp genes that encode a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2.

Brief Summary Text - BSTX:

In other embodiments, humanized gfp genes will encode GFP variants that are generally based upon the foregoing sequence, but that have certain changes. A

particular example is a **humanized gene** that encodes a **GFP** with an amino acid sequence of SEQ ID NO:2 in which Serine at position 65 has been replaced by Threonine.

Brief Summary Text - BSTX:

A further example is a **humanized gfp gene** that encodes a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2 in which Tyrosine at position 66 has been replaced by Histidine.

Brief Summary Text - BSTX:

Another example is a **humanized gfp gene** that encodes a **GFP** that has the amino acid sequence of SEQ ID NO:2 in which the chromophore sequence Phe Ser Tyr Gly Val Gln (SEQ ID NO:4) between positions 64 and 69 has been replaced by the sequence Met Gly Tyr Gly Val Leu (SEQ ID NO:5).

Brief Summary Text - BSTX:

Structural equivalents of the **humanized gfp genes** are also included within the present invention. However, **mutants** that are truncated by more than one amino acid residue at the amino terminus or more than about 10 or 15 amino acid residues from the carboxyl terminus are not generally considered to be useful in the context of producing a fluorescent protein. The encoded **GFP** should therefore be a minimum of about 222 amino acids in length, with proteins of about 238 amino acids in length generally being preferred.

Brief Summary Text - BSTX:

Humanized gfp genes wherein at least about 50% or above of the codon positions contain a humanized codon are also contemplated.

Brief Summary Text - BSTX:

Preferred **humanized gfp genes** of the invention are those genes that contain certain key changes. Examples are **genes that comprises at least seven humanized** codons from the 10 codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the jellyfish **gfp** sequence.

Brief Summary Text - BSTX:

Preferably, **humanized gfp genes will comprise at least eight, at least nine, or ten, humanized** codons from the 10 codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the jellyfish **gfp** gene sequence.

Brief Summary Text - BSTX:

Such constructs are exemplified by humanized genes that comprise any one of the humanized Leucine codons CTG, CTC or TTG at codon positions 18, 53, 125, 178, 195 and 236 of the GFP gene sequence. A further example is a humanized gfp gene that comprises the humanized Valine codon GTG at codon positions 93, 150 and 224 of the GFP gene sequence. Other examples are humanized genes that comprise the humanized Serine codon TCT at codon position 208 of the GFP gene sequence.

Brief Summary Text - BSTX:

The humanized gfp genes encompassed by this invention also include those genes that comprises an increased number of GCC or GCT Alanine-encoding codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO: 1.

Brief Summary Text - BSTX:

By the phrase "increased number of codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1" is meant that the humanized sequence contain an increased number of codons encoding a particular amino acid within the GFP coding region that encodes the amino acid sequence of SEQ ID NO:2, or one of the mutants or other equivalents described herein, in comparison to those codons encoding the same amino acid that are present within the coding region of the wild type jellyfish gene sequence of SEQ ID NO: 1. Thus it will be understood that the term "increased", when used in this context, does not mean the addition of one or more codons to a terminal portion of the coding region, but rather means replacement of an unfavorable codon within the coding region with a codon that is more favorable for translation in a human or mammalian cell.

Brief Summary Text - BSTX:

In light of the definition set forth above, the humanized gfp genes of the invention may also be defined as those genes that comprise an increased number of TGC Cysteine-encoding codons; an increased number of GAC Aspartate-encoding codons; an increased number of GAG Glutamate-encoding codons; an increased number of TTC Phenylalanine-encoding codons; an increased number of GGC Glycine-encoding codons; an increased number of CAC Histidine-encoding codons; an increased number of ATC Isoleucine-encoding codons; an increased number of AAG Lysine-encoding codons; an increased number of CTG or CTC Leucine-encoding codons; an increased number of AAC Asparagine-encoding codons; an increased number of CCC or CCT Proline-encoding codons; an increased number of CAG Glutamine-encoding codons; an increased number of CGC, AGG or CGG Arginine-encoding codons; an increased number of AGC or TCC Serine-encoding codons; an increased number of ACC Threonine-encoding codons; an increased number of GTG or GTC Valine-encoding codons; and/or an increased number of TAC Tyrosine-encoding codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Brief Summary Text - BSTX:

In certain embodiments, the humanized gfp genes may also comprise a TGA termination codon.

Brief Summary Text - BSTX:

Humanized gfp genes may also be defined by comprising a decreased number of certain codons in comparison to the wild type jellyfish gene sequence of SEQ ID NO: 1. "Decreased" in this context also means that the humanized sequence contain a decreased number of codons encoding a particular amino acid within the GFP coding region that encodes the amino acid sequence of SEQ ID NO:2, or a mutant or equivalent thereof, in comparison to those codons encoding the same amino acid that are present within the coding region of the wild type jellyfish gene sequence of SEQ ID NO: 1. Thus it will be understood that "decreased" does not in any way reflect the simple deletion of codons from any portion of the coding region, but again refers to replacement of a jellyfish codon with a codon that occurs more frequently in human genes.

Brief Summary Text - BSTX:

Accordingly, humanized gfp genes of the present invention are also be defined as those genes that comprise a decreased number of GCA Alanine-encoding codons; a decreased number of GGU Glycine-encoding codons; a decreased number of CTT, CTA or TTA Leucine-encoding codons; a decreased number of AGA Arginine-encoding codons; a decreased number of AGT, TCA or TCG Serine-encoding codons; or a decreased number of GTT or GTA Valine-encoding codons.

Brief Summary Text - BSTX:

Although not believed to be required, it is currently preferred that the humanized gfp genes should include a Kozak consensus sequence operatively positioned upstream from the humanized gene sequence (i.e., the gene is positioned downstream from the Kozak consensus sequence).

Brief Summary Text - BSTX:

Certain preferred humanized gfp genes will comprise the nucleic acid sequence of SEQ ID NO:3. However, this is by no means limiting and is just one exemplary embodiment of the present invention. Detailed directions as how to make and use many other such humanized gfp genes are included herein. For example, one may refer to the information in Table 2, Table 3 and Table 4 in creating any one of a number of suitable humanized gfp genes.

Brief Summary Text - BSTX:

Virtually any protein- or peptide-encoding DNA sequence, or combinations thereof, may be fused to a humanized gfp sequence in order to encode a fusion protein. This includes DNA sequences that encode targeting peptides, therapeutic proteins, proteins for recombinant expression, proteins to which one or more targeting peptides is attached, protein subunits and the like.

Brief Summary Text - BSTX:

Recombinant vectors and plasmids form another important aspect of the present invention. In such vectors, the humanized gfp gene is positioned under the transcriptional control of a promoter, generally a promoter operative in a mammalian or human cell. "Positioned under the transcriptional control of" means that the humanized gfp sequence is positioned downstream from and under the transcriptional control of the promoter such the promoter is capable of directing expression of the encoded GFP protein in a mammalian or human host cell upon introduction of the vector into such a cell.

Brief Summary Text - BSTX:

The recombinant vectors of the invention will thus generally comprise a humanized gfp reporter gene operatively positioned downstream from a promoter, wherein the promoter is capable of directing expression of the humanized GFP gene in a mammalian or human cell. Preferably the promoter will direct expression of GFP in an amount sufficient to allow GFP detection by detecting the green fluorescence following expression of GFP in the cell. Such promoters are thus "operative" in mammalian and human cells.

Brief Summary Text - BSTX:

Preferred vectors and plasmids will be constructed with at least one multiple cloning site. In certain embodiments, the expression vector will comprise a multiple cloning site that is operatively positioned between a promoter and a humanized gfp gene sequence. Such vectors may be used, in addition to their uses in other embodiments, to create N-terminal fusion proteins by cloning a second protein-encoding DNA segment into the multiple cloning site so that it is contiguous and in-frame with the humanized gfp sequence.

Brief Summary Text - BSTX:

In other embodiments, expression vectors may comprise a multiple cloning site that is operatively positioned downstream from the expressible humanized gfp gene sequence. These vectors are useful, in addition to their uses, in creating C-terminal fusion proteins by cloning a second protein-encoding DNA segment into the multiple cloning site so that it is contiguous and in-frame with the humanized gfp sequence.

Brief Summary Text - BSTX:

In certain embodiments, the expression vector or plasmid may comprise a humanized GFP reporter gene that has the nucleic acid sequence of SEQ ID NO:3. An exemplary vector is the expression vector termed "pGREENLANTERN.TM."

Brief Summary Text - BSTX:

Reporter gene expression kits are also provided, which kits generally comprise, in suitable container means, at least one expression vector or plasmid that comprises a humanized GFP gene. The vector or plasmid will generally be one that is capable of expressing GFP in an amount sufficient to allow GFP detection by green fluorescence following expression in a mammalian or human cell.

Brief Summary Text - BSTX:

Recombinant host cells form another aspect of the present invention. Such host cells will generally comprise at least one copy of a humanized GFP gene. Preferred cells for expression purposes will be mammalian and human cells. However, it will be understood that other cell types are not excluded from those of the invention. Accordingly, cells such as bacterial, yeast, fungal, insect, nematode and plant cells are also possible, although such cells are not preferred for expression purposes.

Brief Summary Text - BSTX:

In certain embodiments, the recombinant host cells will preferably incorporate a humanized GFP gene in a manner effective to allow the cell to express, or to be stimulated to express, GFP, most preferably, in an amount sufficient to allow GFP detection by its fluorescence. The recombinant host cell will thus preferably include a humanized GFP gene that was introduced into the cell by means of a recombinant vector.

Brief Summary Text - BSTX:

In certain embodiments, the recombinant host cell will express the humanized GFP gene to produce the encoded GFP protein, preferably, in an amount sufficient to allow GFP detection by its fluorescence. It is contemplated that cells containing as few as about 20 copies of a humanized gfp gene will often express the GFP protein in an amount sufficient to allow GFP detection by green fluorescence. In certain embodiments, cells containing as few as about 10 copies, about 5 copies or even about 1 or 2 copies of a humanized gfp gene will also likely satisfy the desired expression criteria, especially where the humanized gfp gene is a mutant gene. In other embodiments, the recombinant host cells may be capable of expressing a humanized gene in order to produce detectable GFP protein within a time frame of about 10 hours, and preferably within about 8 hours, and most preferably within about 6 hours or even less.

Brief Summary Text - BSTX:

Cells of primary cell lines that have been established after removing cells from a mammal and culturing the cells for a limited period of time are also included within the cells of the present invention. These cells may be engineered by the hand of man and returned to the same host animal from which they were originally recovered. Such cells that contain a humanized gfp gene fall within the scope of the invention, irrespective of their location.

Brief Summary Text - BSTX:

Naturally, recombinant cells also include those cells that are located within the body of an animal or human subject, as may have been targeted by gene therapy. These cells include all those that comprise at least one copy of a humanized gfp gene or vector, irrespective of the manner in which gene was acquired, e.g., by transfection, infection and the like.

Brief Summary Text - BSTX:

In certain particular embodiments, recombinant host cells that comprise a humanized GFP gene that comprises the nucleic acid sequence of SEQ ID NO:3 are contemplated.

Brief Summary Text - BSTX:

Many methods of using humanized gfp genes are provided by the present invention. The method of labeling or tagging a mammalian or human cell by expressing at least one humanized GFP gene in the cell is central to each of the methods. The humanized gfp gene should preferably produce GFP in an amount sufficient to allow ready detection of GFP in the cell by detecting GFP fluorescence.

Brief Summary Text - BSTX:

Methods of identifying a mammalian or human cell within a population of cells are also provided. Such methods generally first comprise expressing at least one humanized GFP gene in the cell in a manner effective to produce an amount of GFP sufficient to allow GFP detection by fluorescence. The cell is then admixed, or allowed to become naturally admixed, with a population of cells that do not express GFP, following which the cell is identified by means of identifying a GFP-fluorescent cell.

Brief Summary Text - BSTX:

The term "a GFP-fluorescent cell", as used herein, means that a cell expresses

a humanized GFP gene in a manner effective to result in the production of the GFP product in an amount sufficient to allow subsequent detection of the cell by detecting green fluorescence from GFP in the cell.

Brief Summary Text - BSTX:

The invention further provides methods for identifying a mammalian or human cell that contains an exogenous DNA segment, which methods generally first comprise introducing into a mammalian or human cell an expression vector comprising a humanized GFP gene operatively linked to an exogenous DNA segment. The cell is then preferably cultured under conditions and for a period of time effective to allow expression of the humanized gfp gene in order to produce an amount of GFP sufficient to allow GFP detection by green fluorescence. Subsequently identifying a cell that contains the exogenous DNA segment is then achieved by identifying a GFP-fluorescent cell.

Brief Summary Text - BSTX:

In certain such embodiments, the expression vector for use in such methods will comprise a first coding region defined as the humanized gfp gene that encodes GFP and will also comprise a second coding region that comprises the exogenous DNA segment. These vectors are generally known as vectors that comprises at least two transcriptional or translational units. Two transcriptional units will naturally include two promoters that direct expression of their respective downstream genes.

Brief Summary Text - BSTX:

Two or more humanized gfp genes, each expressing a GFP protein with different spectral properties, may be detected in a cell in the manner described above. GFP-fluorescent cells, whether expressing one, two or more humanized gfp genes, may be identified by a variety of methods, including microscopy and fluorescence activated cell sorting (FACS).

Brief Summary Text - BSTX:

Further examples of methods of the invention are methods for determining the location of a selected protein within a mammalian or human cell. These methods generally comprise first introducing into a cell an expression vector comprising a contiguous DNA sequence comprising a humanized GFP gene operatively linked to a gene encoding said selected protein. The vector will generally express a fusion protein comprising GFP operatively linked to the selected protein, wherein the fusion protein is produced in amounts sufficient to allow cell detection by detecting the green fluorescence of GFP. One can then identify the location of the selected protein within the cell by identifying the location of the green fluorescence from GFP.

Brief Summary Text - BSTX:

Still further examples of methods of the invention are methods for targeting a protein to a selected location within a mammalian or human cell. These methods generally comprise first introducing into the cell an expression vector comprising a DNA sequence comprising a DNA sequence element that encodes a targeting peptide operatively linked and contiguous with a DNA sequence element of a humanized GFP gene, which is also operatively linked and contiguous with a DNA sequence element that encodes a protein. Such vectors express a fusion protein comprising a targeting peptide operatively linked to GFP and to a protein, wherein the fusion protein is produced in the cell in an amount sufficient to allow cell detection by detecting the GFP fluorescence. The protein is then targeted to a selected location within the cell and the location is confirmed by identifying the location of the green fluorescence.

Brief Summary Text - BSTX:

These methods generally comprises introducing into a cell an expression vector comprising a humanized GFP gene under the control of the candidate promoter and maintaining the cell under conditions effective and for a period of time sufficient to allow expression of the humanized GFP gene by the candidate promoter. "Conditions effective" and "periods of time sufficient" are defined as those conditions and times that would ordinarily result in GFP being produced in an amount sufficient to allow GFP detection by green fluorescence when using a known operative promoter.

Brief Summary Text - BSTX:

A further example of methods for using humanized gfp in the context of promoters are those methods for detecting substances that stimulate transcription from a selected promoter in a mammalian or human cell. Again, one generally introduces into a mammalian or human cell an expression vector comprising a humanized GFP gene under the control of a given promoter. One then exposes the cell to a composition suspected of containing a substance known or suspected to be capable of stimulating transcription from the given promoter. The cell is then cultured or maintained for a period of time that would ordinarily allow an active promoter to stimulate GFP-fusion protein production in an amount sufficient to allow cell detection by detecting the GFP-derived green fluorescence. The subsequent identification of a GFP-fluorescent cell is then indicative of the original presence of a substance that stimulates transcription from the given promoter.

Brief Summary Text - BSTX:

These methods are also suitable for use in vitro and in vivo. In vitro uses allow substances such as toxins and pollutants to be detected by using appropriate promoters within the humanized gfp gene constructs.

Brief Summary Text - BSTX:

As part of gene therapy, it is often necessary to determine gene expression levels in the treated mammalian animal or human subject. The present invention also provides methods for determining such the expression levels. These methods generally comprise expressing in cells of the animal an expression vector comprising a **humanized GFP gene** operatively linked to a selected gene. The expression vector will preferably be either a vector that expresses a **GFP**-fusion protein or a vector in which the **humanized gfp gene** and the selected protein gene each use the same or an equivalent promoter. The promoter will have preferably been shown to result in sufficient **GFP** expression to allow detection in vitro. One then determines the GFP-fluorescence in the cells of the animal, wherein the level of **GFP**-fluorescence is indicative of the expression level of the selected gene in the animal.

Brief Summary Text - BSTX:

These methods can be adapted to provide methods for analyzing the expression of a selected gene in different tissues of a mammal or human subject. Such methods generally comprise introducing into the cells of the mammal an expression vector comprising the selected gene under the control of the natural **gene promoter, wherein the gene is operatively linked to a humanized GFP gene.** The vector will preferably express a fusion protein that comprises the encoded gene product operatively linked to **GFP**, the fusion protein being produced in an amount sufficient to allow cell detection by detecting the green fluorescence of **GFP**. After maintaining the mammal under conditions effective and for a period of time sufficient to allow expression of the gene one then analyzes the cells of the tissues of the mammal to detect **GFP**-fluorescent cells, wherein the presence of **GFP**-fluorescent cells in a given tissue is indicative of gene expression in the tissue.

Brief Summary Text - BSTX:

A further example in which the **humanized gfp genes** may be employed is in the recombinant production of **GFP** itself. Such methods of using a **humanized GFP gene simply comprise expressing the humanized gene** in a mammalian or human host cell and collecting the **GFP** expressed by said cell.

Brief Summary Text - BSTX:

(a) preparing a recombinant vector in which a **humanized GFP gene** is positioned under the control of a promoter operative in a mammalian or human cell;

Brief Summary Text - BSTX:

Adaptations of such methods include those wherein the **humanized GFP gene** is fused to a DNA sequence encoding a protein or peptide of known molecular weight. Expression by the host cell thus results in a **GFP** fusion protein that

may be used as a fluorescent molecular weight marker. A range of such fluorescent molecular weight markers could be so-produced to produce a molecular weight determining kit.

Drawing Description Text - DRTX:

FIG. 1A, FIG. 1B and FIG. 1C. Nucleotide sequence of the gfp10 cDNA and the deduced amino acid sequence. Above each codon is the single letter designation for the amino acid. The mutations introduced in the gfp.sub.h sequence are shown below the substituted nucleotide of gfp10. The horizontal lines underline overlap regions of mutually priming oligonucleotides used to synthesize the gfp.sub.h cDNA. The sites of the restriction enzymes used to assemble extended pairs of oligonucleotides are shown in bold letters. The codons mutated to produce the Ser.sub.65 Thr mutation, which produces higher fluorescence yield, and the Tyr.sub.66 His mutation, which produces blue fluorescence, are shown in bold. In FIG. 1A, FIG. 1B and FIG. 1C, the jellyfish gfp10 nucleotide sequence is SEQ ID NO: 1. The deduced amino acid sequence is SEQ ID NO:2. In SEQ ID NO:2, Xaa at position 65 may be Ser or Thr; and Xaa at position 66 may be Tyr or His. The exemplary humanized gfp sequence shown below the substituted nucleotide of gfp10 in FIG. 1A, FIG. 1B and FIG. 1C is SEQ ID NO:3. In SEQ ID NO:3, the nucleotides at positions 193, 195 and 196 may be changed in order to encode either Ser or Thr; and either Tyr or His, as above.

Detailed Description Text - DETX:

For fluorescence microscopy, the inventors increased the sensitivity of the GFP reporter gene system approximately 22 fold for one humanized construct and at least 45 fold for a second humanized construct. In FACS analyses with humanized gene constructs, one construct was at least 32-fold more detectable than the original jellyfish gene, and the other construct was 190-fold more detectable than the original jellyfish gene. When humanized GFP is stably integrated as part of the gfp-neo cassette of the rAAV provirus in G418-resistant cell lines, a considerable portion of the cells express a visually detectable GFP.

Detailed Description Text - DETX:

In addition, the inventors describe the construction of an Ad shuttle vector, carrying the humanized GFP reporter gene under the control of the IRES element. 293 cells infected with recombinant Ad displayed typical CPE and bright green fluorescence. Expression of the GFP allowed for quick and easy selection of true recombinant Ad clones, discriminating them from false plaques.

Detailed Description Text - DETX:

The humanized GFP can also be incorporated into other viral and non-viral vector and expression systems. Using the humanized genes and vectors of the

present invention, efficient transduction and expression of gfp gene sequences in mammalian and human cell lines is possible. This is exemplified by gene expression in vivo within neurosensory cells of guinea pig eye, shown herein. The humanized gfp genes have many uses, such as in cell sorting by fluorescence-activated cell sorting (FACS), and in human gene therapy.

Detailed Description Text - DETX:

Green fluorescent protein genes and functional proteins are believed to be present in a variety of organisms, as shown in Table 1. A gfp gene from any of the bioluminescent cnidaria and ctenophora that express such genes can be used as the starting point for preparing a humanized gfp gene in accordance with the present invention.

Detailed Description Text - DETX:

It is currently preferred that the gfp gene sequence from A. victoria be used as the template for creating a humanized gfp gene, as this is readily available.

Detailed Description Text - DETX:

4. Humanized gfp Genes

Detailed Description Text - DETX:

The foregoing emphasizes the importance of the present invention, the focus of which is to provide for increased GFP expression in mammalian and human cells. Each of the mutants described above, or indeed any desired mutant or a panel of mutants, can also be prepared in a humanized background as provided by the present invention. This is because the humanizing aspects of the invention change the DNA sequence independently of the protein sequence.

Detailed Description Text - DETX:

The approach taken by the present inventors is in contrast to the Adams et. al. (1995) method, and addresses the poor translation efficiency of GFP mRNA in the human cell environment by using cDNAs that contain base substitutions in order to change the codon usage so that it is more appropriate for expression in mammalian cells. Using such humanized constructs results in green fluorescence in cells that have a low copy number of humanized gfp genes, e.g., in the range of less than 10, and even about 1 or 2 when using certain humanized gfp mutant genes.

Detailed Description Text - DETX:

The correlation between the abundance of tRNAs and the occurrence of the respective codons in protein-expressing genes has been described for *E. coli*, yeast and other organisms (Bennetzen and Hall (1982); Grantham et al. (1980); Grantham et al. (1981); Ikemura (1981a; 1981b; 1982); Wada et al. (1990)). However, until codon changes are actually made in any given gene, their effects on translation efficiency and overall expression levels cannot be established. This is similar to the situation involving the Kozak sequence, which is not believed to have been particularly helpful in increasing expression of gfp in mammalian cells despite expectations. Now that the present inventors have shown that humanization is effective for gfp gene expression, the usefulness of the GFP technology has been significantly enhanced.

Detailed Description Text - DETX:

An exemplary humanized sequence in accordance with the present invention is represented by SEQ ID NO:3. However, it will be understood that the humanized sequences of the present invention are by no means limited to the representative sequence of SEQ ID NO:3. Rather, in light of the following instructions, one of skill in the art will readily be able to prepare a number of different humanized gfp sequences.

Detailed Description Text - DETX:

Although any changes that replace a rarely used jellyfish codon with a codon that is more frequently used in human genes are considered to be useful changes, certain codon changes will naturally be preferred over others. In this regard, the inventors have identified a number of gfp codons that are rarely or almost never used in human genes. As discussed below, such codons are the first candidates that should be changed in producing a humanized gene in accordance with the present invention.

Detailed Description Text - DETX:

In making general humanizing changes, codons to be humanized can be identified by those of skill in the art from studying the information presented herein in Tables 2 and in Table 3 and 4. For example, in utilizing the information in Table 2, one would compare the frequency of the jellyfish codon against the frequency of those codons commonly used in human genes, and make any appropriate changes. By way of an example only, consider the amino acid leucine; the codon CUU is used eleven times in the gfp gene, but this codon corresponds to only the fourth preferred codon in human genes. The leucine codon UUA also features prominently in the jellyfish gene, and this codon is the last choice for use in the human genome. Changing the Leucine codons would thus make an appropriate starting point for preparing a humanized gene.

Detailed Description Text - DETX:

From studying the information in Table 3 and Table 4, one of skill in the art

would readily discern that the jellyfish **gfp** codons CTA, TTA, TCG and TCA (or CUA, UUA, UCG or GUA) should be changed to a more preferred codon. As a general guideline, those codons listed in columns 5 and 6 generally represent codons that one would prefer to change in creating a **humanized gene; the codons listed in column 4 should also often be changed in creating a humanized gene**; the codons listed in column 3 may or may not be changed, depending on the number of changes that one wishes to make in total and on the particular amino acid that is to be encoded. Those codons listed in columns 1 and 2, when occurring in the wildtype **gfp** sequence, will generally be appropriate and should not need changing, unless there is only a choice of two codons available. However, replacing a codon from column 2 with a codon from column 1 is certainly a useful option, particularly where there is only a choice of two codons. Given this information, it will now be understood that, when introducing changes into the **gfp** sequence, one would generally desire to introduce a codon of column 1 wherever possible.

Detailed Description Text - DETX:

In light of the foregoing discussion, it will be clear that the exemplary sequence of SEQ ID NO:3 is only one of the many operable species that are encompassed by the present invention. In SEQ. ID NO:3, 88 codons contain one or more base substitutions. 88 codons from a sequence that encodes 328 amino acids represents a change of about 37%. However, it is contemplated that changing about 10% of the codons would produce a useful increase in expression levels and such gene sequences therefore fall within the scope of the present invention. Changing about 15%, 20%, 25% or 30% of the codons within the jellyfish **gfp sequence is also considered to be useful and the humanized genes** of this invention encompass those gene sequences that fall within the aforementioned ranges.

Detailed Description Text - DETX:

In certain embodiments, depending on the nature of the codon changes introduced, it may not be necessary to even make a 10% change in the codon usage of the **gfp** gene. For example, if each of the ten least favored codons were to be changed and replaced with those most preferred for use in human genes, it is contemplated that the resultant sequence may achieve reasonable expression in human and mammalian cells. Changing ten codons from within 328 represents a percentage change of about 4%. Therefore, so-called "4% **humanized genes**" also fall within the scope of the present invention given the following provision--that when making only a limited number of changes, one would generally wish to change the ten codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the **gfp** gene sequence. When making these key changes along with a number of other changes, it is contemplated that changing at least about 7, 8 or 9 of these codons will be sufficient to result in a **humanized gene** with improved expression. As described above, leucine would preferably be encoded by CTG, CTC or TTG; valine would preferably be encoded by GTG; and serine would preferably be encoded by AGC.

Detailed Description Text - DETX:

Although **gfp** gene sequences in which about 4-5, about 10, about 20 or about 30-35% of the codons have been changed will generally be preferred, there is no reason that further changes should not be made if so desired. **Humanized gene sequences in accordance with the present invention may therefore be sequences that contain humanized** codons at about 40%, 50%, 60%, 70% or even about 80-90% of the codon positions within the full length codon region. In reviewing SEQ ID NO:3, with a view to introducing still further humanizing changes, a number of positions are identifiable in which further optimizing changes could be introduced. These include, for example, those codons found at codon positions 6, 9, 14, 17, 19, 21, 23, 26, 27, 31, 33, 34, 35, 36, 40, 45, 50, 51, 62, 71, 83, 99, 101, 102, 111, 115, 116, 128, 130, 132, 133, 134, 136, 142, 157, 171, 173, 174, 181, 183, 186, 209, 210, 213, 223 and 230 of SEQ. ID NO:3.

Detailed Description Text - DETX:

The humanized **GFP** of the present invention renders several of these methods practical rather than speculative. **Humanized gfp genes** can therefore be used to identify transformed cells, e.g., by fluorescence-activated cell sorting (FACS) or fluorescence microscopy; to measure gene expression in vitro and in vivo; to label specific cells in multicellular organisms, e.g., to study cell lineages; to label and locate fusion proteins; and to study intracellular trafficking and the like.

Detailed Description Text - DETX:

In methods to produce fluorescent molecular weight markers, a **humanized gfp gene** sequence is generally fused to one or more DNA sequences that encode proteins having defined amino acid sequences and the fusion proteins are expressed from an expression vector. Expression results in the production of fluorescent proteins of defined molecular weight or weights that may be used as markers (following calculation of the size of the complete amino acid).

Detailed Description Text - DETX:

A first example of this general group is where a **humanized gfp sequence** is fused to a DNA sequence encoding a selected protein in order to directly label the encoded protein with **GFP**. Expressing such a humanized **GFP** fusion protein in a cell results in the production of fluorescently-tagged proteins that can be readily detected. This is useful in simply confirming that a protein is being produced by a chosen host cell. It also allows the location of the selected protein to be determined, whether this represents a natural location or whether the protein has been targeted to an organelle by the hand of man.

Detailed Description Text - DETX:

The **humanized genes** of this invention also provide another dimension to the

analysis of promoters in mammalian cells. As gfp can now be expressed in mammalian and human cells and readily detected, a range of promoters can be tested for their suitability for use with a given gene, cell, or system. This applies to in vitro uses, such as in identifying a suitable promoter for use in recombinant expression and high level protein production, and also in in vivo uses, such as in pre-clinical testing or in gene therapy in human subjects.

Detailed Description Text - DETX:

The use of humanized gfp genes with inducible promoters also extends to an analysis of the promoter itself. An example here is in the analysis of a particular promoter from a group of promoters, such as promoters associated with heat shock proteins, that are known to be expressed in various organisms throughout evolution. In this way, a promoter operable in, for example, yeast, can be taken and expressed in a mammalian cell system in order to determine whether it is operable in mammalian cells and, therefore, to determine whether mammalian cells likely include a homolog of the yeast promoter.

Detailed Description Text - DETX:

In the screening embodiments, the humanized gfp gene will be positioned downstream of a promoter that is known to be inducible by the agent that one wishes to identify. Expression of gfp in the cells will normally be silent, and will be switched on by exposing the cell to a composition that contains the selected agent. In using a promoter that is responsive to, for example, a heavy metal, a toxin, a hormone, a cytokine or other defined molecule, the presence of a heavy metal, toxin, hormone, cytokine or such like can readily be determined.

Detailed Description Text - DETX:

In the biological assays, cells including a humanized gfp gene under the control of a promoter that is inducible by a biological effector molecule may be used to detect the presence of such molecules in various kinds of biological samples, including blood, plasma, semen, urine, saliva and the like. Those effector molecules that are detectable in this way include molecules such as hormones, cytokines, neurotransmitters and the like. Of course, as used throughout this application, it will be understood that the term "promoter" is being used to refer to any regulatory element. Particular examples here are the use of the sterol regulatory element, in conjunction with humanized gfp, to detect sterols in a given composition; and the similar use of the serum response element, which is induced by UV, EGF, PDGF and TPA.

Detailed Description Text - DETX:

In the so-called chemical assays, cells including a humanized gfp gene under the control of a promoter that is inducible by a chemical agent are used to detect the presence of the chemical agent in various compositions. These assays may be used to detect toxins or contaminants in fluids such as drinking

water, and the like. The types of agents that may be detected in this way include heavy metals, toxins and various other pollutants and undesirable chemicals.

Detailed Description Text - DETX:

Humanized gfp genes can be used as one portion of a fusion protein, allowing the location of the protein to be identified. Fusions of GFP with an 'exogenous' protein should preserve both the fluorescence of GFP and functions of the host protein, such as physiological functions and/or targeting functions.

Detailed Description Text - DETX:

Adding a nuclear localization signal to a humanized gfp gene may also be used in order to enhance the fluorescence intensity of the expressed protein by confining the protein to the much smaller space of the nucleus. This is described herein in Example VII in the context of blue GFP mutants.

Detailed Description Text - DETX:

Successful gene therapy generally requires the integration of a gene able to correct the genetic disorder into the host genome, where it would co-exist and replicate with the host DNA and be expressed at a level to compensate for the defective gene. Ideally, the disease would be cured by one or a few treatments, with no serious side effects. There have been several approaches to gene therapy proposed to date, each of which may benefit from combination with the humanized gfp of the present invention.

Detailed Description Text - DETX:

As mentioned earlier, modification and changes may be made in the structure of GFP and still obtain a molecule having like or otherwise desirable characteristics. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of function. It is thus contemplated that various changes may be made in the sequence of humanized gfp proteins, by virtue of changing the underlying DNA, without appreciable loss of their biological utility or activity.

Detailed Description Text - DETX:

Site-specific mutagenesis may be used to prepare further variants of humanized gfp genes. Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants by introducing one or more nucleotide sequence changes into the DNA.

Detailed Description Text - DETX:

In general, site-directed **mutagenesis** in accordance herewith is performed by first obtaining a single-stranded vector or melting apart the two strands of a double stranded vector which includes within its **sequence a DNA sequence which encodes gfp or humanized gfp**. An oligonucleotide primer bearing the desired **mutated** sequence is prepared, generally synthetically, for example by the method of Crea et al. (1978). This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as E. coli polymerase I Klenow fragment, in order to complete the synthesis of the **mutation**-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-**mutated** sequence and the second strand bears the desired **mutation**. This heteroduplex vector is then used to transform appropriate cells, such as E. coli cells, and clones are selected which include recombinant vectors bearing the **mutated** sequence arrangement.

Detailed Description Text - DETX:

The preparation of **sequence variants of the selected humanized gfp gene** using site-directed **mutagenesis** is provided as a means of producing potentially useful **GFP** species and is not meant to be limiting as there are other ways in which sequence variants of **GFP** may be obtained. For example, recombinant vectors encoding the desired **humanized gfp gene** may be treated with **mutagenic** agents to obtain sequence variants (see, e.g., a method described by Eichenlaub, 1979) for the **mutagenesis** of plasmid DNA using hydroxylamine.

Detailed Description Text - DETX:

A wide variety of recombinant plasmids and vectors may be engineered to express a **humanized gfp genes** and so used to deliver **GFP** to a cell.

Detailed Description Text - DETX:

As used herein, the term "expression vector" includes any type of genetic construct containing a nucleic acid **sequence of a humanized gfp gene** in which the nucleic acid sequence is capable of being transcribed in a mammalian or human cell. The expression vectors of the invention should also direct translation into **GFP** protein, as provided by the invention itself. In addition to the **humanized gfp sequence**, expression vectors will generally include restriction enzyme cleavage sites and the other initial, terminal and intermediate DNA sequences that are usually employed in vectors to facilitate their construction and use.

Detailed Description Text - DETX:

For long-term, high-yield production of recombinant proteins, stable expression

is often preferred. Here, rather than using expression vectors that contain viral origins of replication, host cells can be transformed with vectors controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. The combined use of humanized gfp sequences and selectable markers is therefore also contemplated.

Detailed Description Text - DETX:

A "promoter" refers to a DNA sequence recognized by the synthetic machinery of a cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. As used herein, the promoter should be operable in mammalian and human cells. The phrases "operable" and "exerting transcriptional control" mean that the promoter is in the correct location and orientation in relation to the humanized gfp nucleic acid to control RNA polymerase initiation and expression of the humanized gene.

Detailed Description Text - DETX:

The promoter used to express the humanized GFP is not critical to the present invention. In the examples given, the human cytomegalovirus (CMV) immediate early gene promoter has been used (Thomsen et. al., 1984), which results in the constitutive, high-level expression of the foreign gene. However, the use of other viral or mammalian cellular promoters which are well-known in the art is also suitable to achieve expression of the humanized gfp gene.

Detailed Description Text - DETX:

Furthermore, selection of a promoter that is regulated in response to specific chemical or physiological signals can permit inducible expression of the humanized gfp gene. Examples of suitable inducible promoters include the PAI-1, cytochrome P450 gene promoters, heat shock protein genes and hormone inducible gene promoters, and the fos and jun promoters inducible by ionizing radiation.

Detailed Description Text - DETX:

IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. In this manner, multiple genes, one of which will be a humanized gfp gene, can be efficiently expressed using a single promoter/ enhancer to transcribe a single message.

Detailed Description Text - DETX:

In that the vectors for use in these aspects are replication defective, they

will typically not have an adenovirus E1 region. Thus, it will be most convenient to introduce the humanized gfp gene at the position from which the E1 coding sequences have been removed. However, the position of insertion of the humanized gene within the adenovirus sequences is not critical. The humanized transcriptional unit may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described previously by Karlsson et. al. (1986).

Detailed Description Text - DETX:

Expression kits comprising humanized gfp genes form another aspect of the invention. Such kits will generally contain, in suitable container means, a formulation of a humanized gfp gene or a vector capable of expressing a humanized gfp gene. The gene or vector may be provided in a pharmaceutically acceptable formulation.

Detailed Description Text - DETX:

When the components of the kit are provided in one or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. The humanized gfp gene or vector may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, eye dropper, or other such like apparatus, from which the formulation may be applied to a cell, or to an area of the body, or injected into an animal, or applied to and mixed with other components of a kit.

Detailed Description Text - DETX:

The container means will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the humanized gfp gene or vector may be placed, preferably, suitably allocated. A second humanized gfp gene or vector construct may also be provided, wherein the kit will also generally contain a second vial or other container into which this is be placed. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent.

Detailed Description Text - DETX:

The terms "engineered" and "recombinant" cells are intended to refer to a cell into which an exogenous DNA segment or gene that includes a humanized gfp gene sequence has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced exogenous DNA segment or gene. Engineered cells are thus cells having a gene or genes introduced through the hand of man.

Detailed Description Text - DETX:

Primary cells of all vertebrate species are considered for use with the **humanized gfp genes** disclosed herein, whether or not they are returned to the body of an animal. These include, by way of example only, bone marrow cells, nerve cells, lung epithelial cells and hepatocytes.

Detailed Description Text - DETX:

Of course, it will be understood that as the present invention is well suited for use in more direct gene therapy methods, any target cell of the body can contain a **humanized gfp gene** as described in this invention. All such cells are considered to fall within the description of a "recombinant host cell", as used herein. This includes any cell within an animal or human subject that comprises one or more copies of a **humanized gfp gene** or vector, irrespective of the manner in which the cell acquires the gene, e.g., by transfection, infection and the like. Diseased cells, deficient cells and healthy cells are all encompassed within the invention in this manner.

Detailed Description Text - DETX:

Construction of **Humanized GFP Gene** and Vectors

Detailed Description Text - DETX:

This example describes the production of a further **humanized GFP sequences** encoding **GFP** protein variants with different properties to the wild type protein. The variants also have increased expression in mammalian and human cells.

Detailed Description Text - DETX:

Two **mutants** were constructed in the pBS-GFP.sub.h background by site-directed PCR.TM. **mutagenesis**. A first **humanized mutant mirrors the protein sequence** reported by Heim et al. (1995) who described a Ser65 to Thr65 substitution that increased the fluorescence yield in the context of the original jellyfish codon sequence. Reasoning that this **mutation** might be even more effective in the context of the **humanized pTR.sub.BS -UF1 sequence**, the inventors reproduced this point **mutation** in the pTR.sub.BS -UF1 background to produce plasmid pTR.sub.BS -UF2.

Detailed Description Text - DETX:

To compare the expression efficiency of the **humanized gfp constructs with the original jellyfish sequence** the inventors transfected 293 cells with pTR.sub.BS -UF, pTR.sub.BS -UF1, or pTR.sub.BS -UF2 plasmid DNA at various DNA concentrations. The transfected cells were then analyzed by FACS 36 hr after transfection (FIG. 3).

Detailed Description Text - DETX:

Results from these studies revealed that pTR.sub.BS -UF1 carrying the **humanized gfp sequence** consistently produced 5-10 times higher number of cells scored as positive for green fluorescence than the jellyfish sequence. The point **mutation** in pTR.sub.BS -UF2 increased the number of fluorescent cells by an additional 5-10 fold over pTR.sub.BS -UF1.

Detailed Description Text - DETX:

To determine whether the modified **gfp** cDNA was sufficient now to detect the marker gene at low gene copy number, the inventors isolated recombinant AAV viruses by packaging and using the three **gfp** constructs (UF, UF1, and UF2) and used them to transduce the **gfp** marker into 293 cells by virus infection. While there was almost no detectable **GFP** expression from a virus carrying the gfp10 cDNA (rAAV-GFP.sub.J), cells infected with a virus carrying the **humanized gfp.sub.h gene** (rAAV-GFP.sub.H 1, or rAAV-GFP.sub.H 2) were readily detected either visually (FIG. 4A and FIG. 4B), or by FACS analysis. FACS analysis was conducted by harvesting transfected 293 cells and analyzing on a flow cytometer (Becton-Dickinson) equipped for FITC detection at an excitation wavelength of 488 nm. At high M.O.I. (approximately 20) the ratio of infected cells, scored by FACS as fluorescent-positive, reached 70% for rAAV-GFP.sub.H 2.

Detailed Description Text - DETX:

The present example describes the construction of a recombinant adenovirus shuttle plasmid and the construction of recombinant adenovirus expressing **humanized gfp gene**. This exemplifies the use of different vector systems in humanized **GFP** expression.

Claims Text - CLTX:

1. A **humanized green fluorescent protein (GFP) gene**.

Claims Text - CLTX:

2. The **humanized GFP gene** of claim 1, wherein said gene encodes a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2.

Claims Text - CLTX:

3. The **humanized GFP gene** of claim 1, wherein said gene encodes a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2 in which Serine.at position 65 has been replaced by Threonine.

Claims Text - CLTX:

4. The **humanized GFP gene** of claim 1, wherein said gene encodes a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2 in which Tyrosine at position 66 has been replaced by Histidine.

Claims Text - CLTX:

5. The **humanized GFP gene** of claim 1, wherein said gene encodes a green fluorescent protein that has the amino acid sequence of SEQ ID NO:2 in which the chromophore sequence Phe Ser Tyr Gly Val Gln (SEQ ID NO:4) between positions 64 and 69 has been replaced by the sequence Met Gly Tyr Gly Val Leu (SEQ ID NO:5).

Claims Text - CLTX:

6. The **humanized GFP gene of claim 1, wherein at least about 10% of said codon positions contain a humanized** codon.

Claims Text - CLTX:

7. The **humanized GFP gene of claim 6, wherein at least about 15% of said codon positions contain a humanized** codon.

Claims Text - CLTX:

8. The **humanized GFP gene of claim 7, wherein at least about 20% of said codon positions contain a humanized** codon.

Claims Text - CLTX:

9. The **humanized GFP gene of claim 8, wherein at least about 25% of said codon positions contain a humanized** codon.

Claims Text - CLTX:

10. The **humanized GFP gene of claim 9, wherein at least about 30% of said codon positions contain a humanized** codon.

Claims Text - CLTX:

11. The **humanized GFP gene of claim 10, wherein at least about 35% of said codon positions contain a humanized** codon.

Claims Text - CLTX:

12. The humanized GFP gene of claim 11, wherein at least about 50% of said codon positions contain a humanized codon.

Claims Text - CLTX:

13. The humanized GFP gene of claim 1, wherein said gene comprises at least seven humanized codons from the 10 codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the GFP gene sequence.

Claims Text - CLTX:

14. The humanized GFP gene of claim 13, wherein said gene comprises at least eight humanized codons from the 10 codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the GFP gene sequence.

Claims Text - CLTX:

15. The humanized GFP gene of claim 14, wherein said gene comprises at least nine humanized codons from the 10 codons located at codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the GFP gene sequence.

Claims Text - CLTX:

16. The humanized GFP gene of claim 15, wherein said gene comprises a humanized codon at each of the codon positions 18, 53, 93, 125, 150, 178, 195, 208, 236 and 224 of the GFP gene sequence.

Claims Text - CLTX:

17. The humanized GFP gene of claim 13, wherein said gene comprises any one of the humanized Leucine codons CTG, CTC or TTG at codon positions 18, 53, 125, 178, 195 and 236 of the GFP gene sequence.

Claims Text - CLTX:

18. The humanized GFP gene of claim 13, wherein said gene comprises the humanized Valine codon GTG at codon positions 93, 150 and 224 of the GFP gene sequence.

Claims Text - CLTX:

19. The humanized GFP gene of claim 13, wherein said gene comprises the

humanized Serine codon TCT at codon position 208 of the GFP gene sequence.

Claims Text - CLTX:

20. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of GCC or GCT Alanine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

21. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of TGC Cysteine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

22. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of GAC Aspartate-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

23. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of GAG Glutamate-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

24. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of TTC Phenylalanine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

25. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of GGC Glycine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

26. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of CAC Histidine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

27. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of ATC Isoleucine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

28. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of AAG Lysine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

29. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of CTG or CTC Leucine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

30. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of AAC Asparagine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

31. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of CCC or CCT Proline-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

32. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of CAG Glutamine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

33. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of CGC, AGG or CGG Arginine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

34. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of AGC or TCC Serine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

35. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of ACC Threonine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

36. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of GTG or GTC Valine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

37. The **humanized GFP gene** of claim 1, wherein said gene comprises an increased number of TAC Tyrosine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

38. The **humanized GFP gene** of claim 1, wherein said gene comprises a TGA termination codon.

Claims Text - CLTX:

39. The **humanized GFP gene** of claim 1, wherein said gene comprises a decreased number of GCA Alanine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

40. The **humanized GFP gene** of claim 1, wherein said gene comprises a decreased number of GGU Glycine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

41. The **humanized GFP gene** of claim 1, wherein said gene comprises a decreased number of CTT, CTA or TTA Leucine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

42. The **humanized GFP gene** of claim 1, wherein said gene comprises a decreased

number of AGA Arginine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

43. The **humanized GFP gene** of claim 1, wherein said gene comprises a decreased number of AGT, TCA or TCG Serine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

44. The **humanized GFP gene** of claim 1, wherein said gene comprises a decreased number of GTT or GTA Valine-encoding codons within the coding region in comparison to the wild type jellyfish gene sequence of SEQ ID NO:1.

Claims Text - CLTX:

45. The **humanized GFP gene** of claim 1, wherein said gene is operatively positioned downstream from a Kozak consensus sequence.

Claims Text - CLTX:

46. The **humanized GFP gene** of claim 1, wherein said gene comprises the nucleic acid sequence of SEQ ID NO:3.

Claims Text - CLTX:

47. The **humanized GFP gene** of claim 1, wherein said gene is operatively linked to protein-encoding nucleic acid sequence.

Claims Text - CLTX:

48. The **humanized GFP gene** of claim 1, wherein said gene is positioned under the transcriptional control of a promoter operative in a mammalian cell.

Claims Text - CLTX:

49. The **humanized GFP gene** of claim 48, further defined as a recombinant vector.

Claims Text - CLTX:

50. An expression vector comprising a **humanized GFP reporter gene operatively positioned downstream from a promoter, the promoter directing expression of the**

humanized GFP gene in a mammalian cell.

Claims Text - CLTX:

60. The expression vector of claim 59, wherein said expression vector comprises a multiple cloning site operatively positioned between said promoter and said **humanized GFP gene**.

Claims Text - CLTX:

61. The expression vector of claim 59, wherein said expression vector comprises a multiple cloning site operatively positioned downstream from said **humanized GFP gene**.

Claims Text - CLTX:

70. The expression vector of claim 50, wherein said expression vector comprises a **humanized GFP reporter gene** that has the nucleic acid sequence of SEQ ID NO:3.

Claims Text - CLTX:

71. A recombinant host cell comprising a **humanized GFP gene**.

Claims Text - CLTX:

72. The recombinant host cell of claim 71, wherein said **humanized GFP gene** is introduced into said cell by means of a recombinant vector.

Claims Text - CLTX:

73. The recombinant host cell of claim 72, wherein said cell expresses said **humanized GFP gene** to produce the encoded **GFP** protein.

Claims Text - CLTX:

79. The recombinant host cell of claim 71, wherein said cell comprises a **humanized GFP gene** that comprises the nucleic acid sequence of SEQ ID NO:3.

Claims Text - CLTX:

81. A reporter **gene expression kit comprising, in suitable container means, an expression vector comprising a humanized GFP gene**.

US-PAT-NO: 5869035

DOCUMENT-IDENTIFIER: US 5869035 A

TITLE: Methods and compositions for inducing complement destruction of tissue

DATE-ISSUED: February 9, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|-----------------|-------|----------|---------|
| Link, Jr.; Charles J. | Clive | IA | N/A | N/A |
| Levy; John P. | West Des Moines | IA | N/A | N/A |

APPL-NO: 08/ 748344

DATE FILED: November 13, 1996

US-CL-CURRENT: 424/93.7; 424/277.1 ; 424/93.21 ; 435/320.1 ; 514/44

ABSTRACT:

The invention discloses methods and compositions for killing tumor cells in animals. Through transfer techniques, cancer cells are engineered to express an epitope which is targeted by natural antibodies causing complement destruction of transformed tumor cells that is typically associated with hyperacute xenograft rejection.

14 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Drawing Description Text - DRTX:

FIG. 4 is a photograph depicting lymphocytes transduced by murine LNChRG retroviral vector expressing a **humanized, red shifted GFP mutant gene**. Lymphocytes were transduced by phosphate depletion method. The living cells were visualized with an FITC filter at 100.times. magnification

Detailed Description Text - DETX:

Efficient retroviral transduction of lymphocytes is the first requirement for an ex vivo approach using the .alpha.((1,3)GT gene to ablate GvHD inducing T-cells. Our laboratory has previously developed a protocol for adoptive

immunotherapy using retroviral gene transfer of the HStk gene. The problems with the HStk gene transfer approach are two fold. First, the transduced lymphocytes require drug selection after transduction with neomycin to eliminate non-transduced cells and the selection can damage T-cells (data not shown). The second problem is that some patients who have completed an allogeneic bone marrow transplant develop significant infections with Cytomegalovirus (CMV) or HSV that requires treatment with GCV or acyclovir. If such infections occur, the anti-viral therapy would destroy the adoptively transferred gene modified lymphocytes. Since recurrent leukemia patients (especially CML patients) have a substantial response rate to adopted lymphocytes that may be curative, premature destruction of donor lymphocytes in the absence of substantial or refractory GvHD may be harmful to the patient. For this project we propose solutions to these two concerns. To permit lymphocyte sorting without drug selection, our vector will contain a humanized, red-shifted green fluorescent protein (hRGFP) gene (See FIG. 4). The second major modification is the use of doxycycline inducible promoter (dip) for control of the $\alpha(1,3)$ GT gene expression. The dip regulatory cassette will be incorporated into a retroviral vector. We previously demonstrated the results of FACS sorting of hRGFP expressing tumor cells. Approximately a 3 log increase in mean green fluorescent intensity was noted with expression from the retroviral construct. This shift will make it straight forward to sort GFP gene modified lymphocytes as well. We have also previously transduced human lymphocytes with the HStk gene and demonstrated that they can be inhibited by GCV. Under optimal conditions, greater than 90% of the HStk transduced and selected lymphocytes can be destroyed.

Detailed Description Text - DETX:

The diP expression cassette (kindly provided by Dr. Reeves, Mass. Gen Hospital) contains a mutated tetracycline repressor (mtetR) expressed from CMV immediate early promoter. The modified tetR gene contains amino acid substitutions that result in binding of doxycycline (DCN) and then activation of the tetracycline responsive element (TRE) promoter region. The TRE element is fused to a truncated CMV promoter. Binding to the TRE element by the doxycycline and mtetR protein complex results in the induction of high level gene expression. The $\alpha(1,3)$ GT gene will be cloned just downstream of this promoter. The final vector once integrated and expressed in target lymphocytes should exhibit induced expression of $\alpha(1,3)$ GT protein and subsequent presentation of $\alpha(1,3)$ galactosyl epitopes on the cell surface. The $\alpha(1,3)$ GT gene will be PCR amplified from the pL α .SN vector to include Sac II and Xba I restriction sites at the 5' and 3' end of the gene's open reading frame respectively. This PCR product will be restriction digested with Sac II and Xba I and cloned into the Sac II and Xba I site of plasmid pTRE (Clontech Corp., Palo Alto, Calif.) to obtain plasmid pTRE. α . Plasmid pTet-on containing the mtetR and VP16 fusion gene under control of the CMV promoter will be PCR amplified from the plasmid to contain flanking Xba I sites and then cloned into the corresponding site of plasmid pTRE. α . Restriction analysis and sequencing will be done to obtain intact mtetR gene inserts in both the 5' to 3' and 3' to 5' orientation to obtain pT. α .di5 and pT. α .di3 respectively. The doxycycline inducible cassette will next be cloned into plasmid pLhRGSN (provided by R. Muldoon, HGTRI, IA). This plasmid contains the same red shifted, humanized hRGFP gene in the LXSN retroviral

backbone. The .alpha.(1,3)GT expression cassettes from both the pT.alpha.di5 and pT.alpha.di3 plasmid will be cloned downstream of the hRGFP gene at the available Xba I site and blunt end ligated to the 3' LTR sequence. The resulting plasmids pLGT.alpha.di5 and pLGT.alpha.di3 will have constitutive GFP expression from the Maloney virus LTR and doxycycline inducible expression of .alpha.(1,3)GT.

US-PAT-NO: 5820868

DOCUMENT-IDENTIFIER: US 5820868 A

TITLE: Recombinant protein production in bovine adenovirus expression vector system

DATE-ISSUED: October 13, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|------------|-------|----------|---------|
| Mittal; Suresh K. | Saskatoon | N/A | N/A | CA |
| Graham; Frank L. | Hamilton | N/A | N/A | CA |
| Prevec; Ludvik | Burlington | N/A | N/A | CA |
| Babiuk; Lorne A. | Saskatoon | N/A | N/A | CA |

APPL-NO: 08/ 164292

DATE FILED: December 9, 1993

US-CL-CURRENT: 424/199.1; 424/233.1 ; 435/235.1 ; 435/320.1

ABSTRACT:

The present invention relates novel live bovine adenovirus (BAV) expression vector systems in which part or all of one or both of the early region 1 (E1) and early region 3 (E3) genes are deleted and replaced by a foreign gene or fragment thereof and novel recombinant mammalian cell lines stably transformed with BAV E1 sequences, and therefore, express E1 gene products capable of allowing replication therein of a bovine adenovirus having an E1 deletion replaced by a heterologous nucleotide sequence encoding a foreign gene or fragment thereof and their use in production of (antigenic) polypeptides or fragments thereof for the purpose of live recombinant virus or subunit vaccine or for other therapies.

5 Claims, 58 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 51

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Detailed Description Text - DETX:

A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by

a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, viral DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Detailed Description Text - DETX:

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a viral gene, the gene will usually be flanked by DNA that does not flank the viral gene in the genome of the source virus or virus-infected cells. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

Detailed Description Text - DETX:

Luciferase was expressed as an active enzyme as determined by luciferase assays using extracts from MDBK cells-infected with BAV3-Luc (see FIG. 13). The luciferase gene without any exogenous regulatory sequences was inserted into E3 of the BAV3 genome, therefore, there was a possibility of luciferase expression as a fusion protein with part of an E3 protein if the luciferase gene was in the same frame, Such as, F1 and F3 which represent open reading frames (ORFs) for E3 proteins (FIG. 15) or the fusion protein may arise due to recognition of an upstream initiation codon in the luciferase ORF. To explore this possibility we sequenced the DNA at the junction of the luciferase gene and the BAV3 sequences with the help of a plasmid, pSM51-Luc and a synthetic primer design to bind luciferase coding sequences near the initiation codon (data not shown). The luciferase coding region fell in frame F2. The luciferase initiation codon was the first start codon in this frame, however, the ORF started at 84 nucleotides upstream of the luciferase start codon. To further confirm that luciferase protein is of the same molecular weight as purified firefly luciferase, unlabeled mock infected, wt BAV3-infected or BAV3-Luc-infected MDBK cell extracts were reacted with an anti-luciferase antibody in a Western blot (FIG. 16). A 62 kDa polypeptide band was visible in the BAV3-Luc (lane 3 and 4)-infected cell extracts which were of the same molecular weight as pure firefly luciferase (lane 5). We are not sure whether a band of approximately 30 kDa which also reacted with the anti-luciferase antibody in lanes 3 and 4 represented a degraded luciferase protein.

US-PAT-NO: 5795737

DOCUMENT-IDENTIFIER: US 5795737 A

TITLE: High level expression of proteins

DATE-ISSUED: August 18, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------|-------------|-------|----------|---------|
| Seed; Brian | Boston | MA | N/A | N/A |
| Haas; Jurgen | Schriesheim | N/A | N/A | DE |

APPL-NO: 08/ 532390

DATE FILED: September 22, 1995

PARENT-CASE:

This application is a continuation-in-part of allowed application U.S. Ser. No. 08/324,243, filed Sep. 19, 1994, hereby incorporated by reference.

US-CL-CURRENT: 435/69.1; 435/183 ; 435/252.3 ; 435/254.11 ; 435/254.2 ; 536/23.1 ; 536/23.5

ABSTRACT:

The invention features a synthetic gene encoding a protein normally expressed in a mammalian cell or eukaryotic cell wherein at least one non-preferred or less preferred codon in the natural gene encoding the mammalian protein has been replaced by a preferred codon encoding the same amino acid.

14 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Detailed Description Text - DETX:

To compare the wild-type and **synthetic gp120 coding sequences, the synthetic gp120 coding sequence** was inserted into a mammalian expression vector and tested in transient transfection assays. Several different native gp120 genes were used as controls to exclude variations in expression levels between different virus isolates and artifacts induced by distinct leader sequences.

The gp120 HIV IIIb construct used as control was generated by PCR using a Sal1/Xho1 HIV-1 HXB2 envelope fragment as template. To exclude PCR induced **mutations**, a Kpn1/EarI fragment containing approximately 1.2 kb of the gene was exchanged with the respective sequence from the proviral clone. The wild-type gp120mn constructs used as controls were cloned by PCR from HIV-1 MN infected C8166 cells (AIDS Repository, Rockville, Md.) and expressed gp120 either with a native envelope or a CD5 leader sequence. Since proviral clones were not available in this case, two clones of each construct were tested to avoid PCR artifacts. To determine the amount of secreted gp120 semi-quantitatively supernatants of 293T cells transiently transfected by calcium phosphate co-precipitation were immunoprecipitated with soluble CD4:immunoglobulin fusion protein and protein A sepharose.

Detailed Description Text - DETX:

To examine whether **regulation** by rev is connected to HIV-1 codon usage, the influence of rev on the expression of both native and **synthetic gene** was investigated. Since **regulation** by rev requires the rev-binding site RRE in cis, constructs were made in which this binding site was cloned into the 3' untranslated region of both the native and the **synthetic gene**. These plasmids were co-transfected with rev or a control plasmid in trans into 293T cells, and gp120 expression levels in supernatants were measured semiquantitatively by immunoprecipitation. The procedures used in this experiment are described in greater detail below.

Detailed Description Text - DETX:

As shown in FIG. 5, panel A and FIG. 5, panel B, rev up **regulates** the native gp120 **gene, but has no effect on the expression of the synthetic gp120 gene**. Thus, the action of rev is not apparent on a substrate which lacks the coding sequence of endogenous viral envelope sequences.

Detailed Description Text - DETX:

The above-described experiment suggest that in fact "envelope sequences" have to be present for rev **regulation**. In order to test this hypothesis, a **synthetic version of the gene** encoding the small, typically highly expressed cell surface protein, rat THY-1 antigen, was prepared. The **synthetic version of the rat THY-1 gene** was designed to have a codon usage like that of HIV gp120. In designing this **synthetic gene** AUUUA sequences, which are associated with mRNA instability, were avoided. In addition, two restriction sites were introduced to simplify manipulation of the resulting gene (FIG. 6). This **synthetic gene** with the HIV envelope codon usage (rTHY-1env) was generated using three 150 to 170 mer oligonucleotides (FIG. 7). In contrast to the syngp120 mn gene, PCR products were directly cloned and assembled in pUC12, and subsequently cloned into pCDM7.

Detailed Description Text - DETX:

Examination of a codon usage table constructed from the native coding sequence of **GFP** showed that the **GFP** codons favored either A or U in the third position. The bias in this case favors A less than does the bias of gp120, but is substantial. A **synthetic gene** was created in which the natural **GFP** sequence was re-engineered in much the same manner as for gp120. The **sequence of this synthetic GFP gene**, having its translational start at nucleotide 28, is depicted in FIG. 11 (SEQ ID NO:40). In addition, the translation initiation sequence of **GFP** was replaced with sequences corresponding to the translational initiation consensus. The expression of the resulting protein was contrasted with that of the wild type sequence, similarly engineered to bear an optimized translational initiation consensus (FIG. 10, panel B and FIG. 10, panel C, respectively). In addition, the effect of inclusion of the **mutation** Ser 65.fwdarw.Thr, reported to improve excitation efficiency of **GFP** at 490 nm and hence preferred for fluorescence microscopy (Heim et al., Nature 373:663,1995), was examined (FIG. 10, panel D). Codon engineering conferred a significant increase in expression efficiency (an concomitant percentage of cells apparently positive for transfection), and the combination of the Ser 65.fwdarw.Thr **mutation** and codon optimization resulted in a DNA segment encoding a highly visible mammalian marker protein (FIG. 10, panel D).

Detailed Description Text - DETX:

The above-described **synthetic green fluorescent protein coding sequence** was assembled in a similar manner as for gp120 from six fragments of approximately 120 bp each, using a strategy for assembly that relied on the ability of the restriction enzymes BsaI and BbsI to cleave outside of their recognition sequence. Long oligonucleotides were synthesized which contained portions of the coding sequence for **GFP** embedded in flanking sequences encoding EcoRI and BsaI at one end, and BamHI and BbsI at the other end. Thus, each oligonucleotide has the configuration EcoRI/BsaI/GFP fragment/BbsI/BamHI. The restriction site ends generated by the BsaI and BbsI sites were designed to yield compatible ends that could be used to join adjacent **GFP** fragments. Each of the compatible ends were designed to be unique and non-selfcomplementary. The crude synthetic DNA segments were amplified by PCR, inserted between EcoRI and BamHI in pUC9, and sequenced. Subsequently the intact coding sequence was assembled in a six fragment ligation, using insert fragments prepared with BsaI and BbsI. Two of six plasmids resulting from the ligation bore an insert of correct size, and one contained the desired full length sequence. **Mutation** of Ser65 to Thr was accomplished by standard PCR based **mutagenesis**, using a primer that overlapped a unique BssSI site in the synthetic **GFP**.

Detailed Description Text - DETX:

Synthetic GFP genes can be used in any application in which a native GFP gene **or** other reporter gene can be used. A synthetic **GFP gene which employs** more preferred codons than the native GFP gene **can** be the basis of a highly sensitive reporter system. Such a system can be used, e.g., to analyze the influence of particular promoter elements or trans-acting factors on gene expression. Thus, the synthetic **GFP gene can be used** in much the same fashion

as other reporters, e.g., β -galactosidase, has been used.